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(57) Abstract

The present invention relates to diagnostic tools for veterinary and human use which are used for serodiagnosing ehrlichiosis in mammals, particularly in members of the Canidae family and in humans. The present invention also provides polynucleotides which encode the outer membrane proteins of E. chafeensis. The polynucleotides encode an OMP-1 family of proteins of E. chafeensis and P30 family of proteins of E. canis. The present invention also provides the following isolated proteins of E. chafeensis OMP-1, OMP-1A, OMP-1B, OMP-1C, OMP-1D, OMP-1E, OMP-1F, OMP-1R, OMP-1S, OMP-1T, OMP-1U, OMP-1V, OMP-1W, OMP-1X, and OMP-1Z, referred to hereinafter collectively as the "OMP family". The present invention also provides the following isolated proteins of E. canis P30, P30-a, P30-1, P30-2, P30-3, P30-4, P30-5, P30-6, P30-7, P30-8, P30-9, and P30-10, referred to hereinafter as the P30 family. The present invention also relates to an assay for diagnosing ehrlichiosis in humans using a recombinant outer membrane protein of E. chafeensis, particularly OMP-1. The present invention also relates to an assay for diagnosing ehrlichiosis in humans and members of the family Canidae using a recombinant outer membrane protein of E. canis, particularly P30.

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OUTER MEMBRANE PROTEIN OF EHRLICHIA CANIS AND EHRLICHIA CHAFFEENIS

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BACKGROUND OF THE INVENTION

The ehrlichiae are obligate intracellular bacteria that infect circulating leucocytes. *Ehrlichia chafeensis* infects the monocytes and macrophages in humans and causes human monocytic ehrlichiosis. The clinical manifestations of ehrlichiosis in humans are nonspecific and similar to Rocky Mountain spotted fever. The clinical manifestations include fever, chills, headache mylagia or vomiting and weight loss. Most patients have a history of tick exposure.

Ehrlichia canis infects and causes ehrlichiosis in animals belonging to the family Canidae. Canine ehrlichiosis consists of an acute and a chronic phase. The acute phase is characterized by fever, serous nasal and ocular discharges, anorexia, depression, and loss of weight. The chronic phase is characterized by severe pancytopenia, epistaxis, hematuria, blood in feces in addition to more severe clinical signs of the acute disease. If treated early during the course of the disease, dogs respond well to doxycycline. However, chronically infected dogs do not respond well to the antibiotic. Therefore, early diagnosis is very important for treating canine ehrlichiosis.

The primary diagnostic test for diagnosing canine ehrlichiosis and human ehrlichiosis is the indirect fluorescent antibody (IFA) test. This test uses the etiologic agent *Ehrlichia canis* to diagnose canine ehrlichiosis. The IFA test uses *Ehrlichia chafeensis* as antigen for diagnosing human ehrlichiosis. The IFA test has, however, serious limitations. The IFA test is subject to false positives because the antigens are made of whole infected cells which comprise many nonspecific proteins which will cross-react with sera from some patients. The IFA test is also subject to false negatives because IFA antigens are unstable and may become inactivated during storage. In addition the IFA test requires a special equipment to perform the test. For example, the IFA test requires a tissue culture system for growing the bacterium that are used to prepare the antigen slides, a fluorescent microscope, and trained persons to evaluate the serum reactivity to the bacterial antigen on the slide.

Tools which permit simpler, more rapid, and objective serodiagnosis of canine ehrlichiosis or human ehrlichiosis are desirable.

SUMMARY OF THE INVENTION

The present invention relates to improved diagnostic tools for veterinary and human use which are used for serodiagnosing ehrlichiosis in mammals, particularly in members of the Canidae family and in humans.

The present invention also provides polynucleotides or nucleic acids which encode the outer membrane proteins of E. chafeensis. The OMP-1 polynucleotide encodes an OMP-1 protein of E. chafeensis having a molecular weight of about 27.7 kDa and an amino acid sequence which is at least 85% homologous to the amino acid sequence shown in FIG.3B, SEQ ID NO: __. The OMP-1B polynucleotide encodes an OMP-1B protein of E.

chafeensis having a molecular weight of about 28.2 kDa and an amino acid sequence which is at least 85% homologous to the amino acid sequence shown in FIG. 4B, SEQ ID NO: __. The OMP-1C polynucleotide encodes an OMP-1C protein of E. chafeensis having a molecular weight of about 27.6 kDa and an amino acid sequence which is at least 85% homologous to the amino acid sequence shown in FIG. 5B, SEQ ID NO: __. The OMP-1D polynucleotide encodes an OMP-1D protein of E. chafeensis having a molecular weight of about 28.7 kDa and an amino acid sequence which is at least 85% homologous to the amino acid sequence shown in FIG. 6B, SEQ ID NO: __. The OMP-1E polynucleotide encodes an OMP-1E protein of E. chafeensis having a molecular weight of about 27.8 kDa and an amino acid sequence which is at least 85% homologous to the amino acid sequence shown in FIG. 7B, SEQ ID NO: __. The OMP-1F polynucleotide encodes an OMP-1F protein of E. chafeensis having a molecular weight of about 27.9 kDa and an amino acid sequence which is at least 85% homologous to the amino acid sequence shown in FIG. 8B, SEQ ID NO: __. The OMP-1A polynucleotide encodes an OMP-1A protein of E. chafeensis having a molecular weight of about 29.6 kDa and an amino acid sequence which is at least 85% homologous to the amino acid sequence shown in FIG. 9B, SEQ ID NO: __. The OMP-1R polynucleotide encodes an OMP-1R protein of E. chafeensis having a molecular weight of at least 23 kDa and comprising an amino acid sequence which is at least 85% homologous to the amino acid sequence shown in FIG. 10B, SEQ ID NO: __. The OMP-1S polynucleotide encodes an OMP-1S protein of E. chafeensis comprising an amino acid sequence which is at least 85% homologous to the amino acid sequence shown in FIG. 11B, SEQ ID NO: __. The OMP-1T polynucleotide encodes an OMP-1T protein of E. chafeensis comprising an amino acid sequence which is at least 85% homologous to the amino acid sequence shown in FIG. 12B, SEQ ID NO: __. The OMP-1U polynucleotide encodes an OMP-1U protein of E. chafeensis having a molecular weight of about 30.6 kDa and an amino acid sequence which is at least 85% homologous to amino acid sequence shown in FIG. 13B, SEQ ID NO: __. The OMP-1V polynucleotide encodes an OMP-1V protein of E. chafeensis having a molecular weight of about 28.0 kDa and an amino acid sequence which is at least 85% homologous to the amino acid sequence shown in FIG. 14B, SEQ ID NO: __. The OMP-1W polynucleotide encodes an OMP-1W protein of E. chafeensis having a molecular weight of about 28.8 kDa and an amino acid sequence which is at least 85% homologous to the amino acid sequence shown in FIG. 15B, SEQ ID NO: __. The OMP-1X polynucleotide encodes an OMP-1S protein of E. chafeensis having a molecular weight of about 27.8 kDa and an amino acid sequence which is at least 85% homologous to the amino acid sequence shown in FIG. 16B, SEQ ID NO: __. The OMP-1Y polynucleotide encodes an OMP-1Y protein of E. chafeensis having a molecular weight about 28.8 kDa and an amino acid sequence which is at least 85% homologous to the amino acid sequence shown in FIG. 17B, SEQ ID NO: __. The OMP-1Z polynucleotide encodes an OMP-1Z protein of E. chafeensis comprising an amino acid sequence which is at least 85% homologous to the amino acid sequence shown in FIG. 18B, SEQ ID NO: __.

The outer membrane proteins from E. chaffeensis, particularly a recombinant form of OMP-1, are immunogenic and, thus are useful for preparing antibodies. Such antibodies are useful for immunolabeling isolates of E. chafeensis and for detecting the presence of E. chafeensis in body fluids, tissues, and particularly in monocytes and macrophages. The isolated outer membrane proteins, particularly OMP-1, are also useful for

detecting antibodies to E. chafeensis in the blood of patients with clinical signs of ehrlichiosis. The isolated outer membrane protein, particularly OMP-1, are also useful immunogens for raising antibodies that are capable of reducing the level of infection in an immunized mammal that has been infected with E. chafeensis. The isolated membrane proteins are also useful in a vaccine for protecting against infection with E. chafeensis.

The present invention also relates to isolated polynucleotides which encode 30 kDa outer membrane proteins from Ehrlichia canis. The proteins are designated P30 and P30a. The proteins, particularly P30, are immunogenic and are, thus, useful for preparing antibodies that are useful for immunolabeling isolates of E. canis. The P30 protein is also useful for diagnosing canine ehrlichiosis in mammals, particularly in members of the family Canidae, most particularly in dogs and for diagnosing infections with E. chafeensis in humans. The P30 protein is also a useful immunogen for raising antibodies that reduce the level of infection in an immunized mammal that has been infected with E. canis. The P30 protein is also useful in a vaccine for protecting animals against infection with E. canis.

The present invention also provides the following isolated proteins of E. chafeensis OMP-1 (also known as p28), OMP-1A, OMP-1B, OMP-1C, OMP-1D, OMP-1E, OMP-1F, OMP-1R, OMP-1S, OMP-1T, OMP -1U, OMP-1V, OMP-1W, OMP-1X, and OMP-1Z, referred to hereinafter collectively as the "OMP family". The present invention also provides the following isolated proteins of E. canis P30, P30-a, P30-1, P30-2, P30-3, P30-4, P30-5, P30-6, P30-7, P30-8, P30-9, and P30-10, referred to hereinafter as the P30 family.

The present invention also relates to an assay for diagnosing ehrlichiosis in humans using a recombinant outer membrane protein of E. chafeensis, particularly OMP-1. The present invention also relates to an assay for diagnosing ehrlichiosis in humans and members of the family Canidae using a recombinant outer membrane protein of E. canis, particularly P30.

Brief Description of the Figures

FIG. 1. shows the DNA sequence of and the amino acid sequence encoded by the *E. chafeensis (p28)* gene cloned in pCRII*p28*. The N-terminal amino acid sequence of native *omp-1* protein (P28) determined chemically is underlined. Five amino acid residues at the N terminus of P28 which were not included in the *p28* gene, are indicated by boldface. Arrows indicate annealing positions of the primer pair designed for PCR

FIG. 2. shows the restriction map of 6.3-kb genomic DNA including the *omp-1* gene copies in *E. chafeensis*. The four DNA fragments were cloned from the genomic DNA (pPS2.6, pPS3.6, pEC2.6, and pEC3.6). A recombinant plasmid pPS2.6 has an overlapping sequence with that of pEC3.6. The closed boxes at the bottom show PCR-amplified fragments from the genomic DNA for confirmation of the overlapping area. Open boxes at the top indicate open reading frames (ORF) of *omp-1* gene copies with direction by arrows. Open boxes at the bottom show DNA fragments subcloned for DNA sequencing.

FIG. 3B shows one embodiment of the OMP-1 protein; FIG. 3A shows one embodiment of the OMP-1 polynucleotide.

FIG. 4B shows one embodiment of the OMP-1B protein, FIG. 4A shows one embodiment of the OMP-1B polynucleotide

FIG. 5A shows one embodiment of the OMP-1C polynucleotide; FIG 5B shows one embodiment of the OMP-1C protein.

FIG. 6B shows one embodiment of the OMP-1D protein; FIG. 6A shows one embodiment of the OMP-1D polynucleotide.

FIG. 7A shows one embodiment of the OMP-1E protein; FIG 7B shows one embodiment of the OMP-1E polynucleotide.

FIG. 8A shows one embodiment of the OMP-1F protein; FIG 8 B shows one embodiment of the OMP-1F polynucleotide.

FIG. 9B shows one embodiment of the OMP-1A protein, FIG 9A shows one embodiment of the OMP-1A polynucleotide.

FIG. 10 B shows one embodiment of a portion of the OMP-1R protein, FIG 10A shows one embodiment of an OMP-1R polynucleotide encoding such polypeptide.

FIG. 11 B shows one embodiment of a portion of the OMP-1S protein, FIG 11A shows one embodiment of the OMP-1S polynucleotide encoding such polypeptide.

FIG. 12 B shows one embodiment of a portion of the OMP-1T protein, FIG 12A shows one embodiment of the OMP-1T polynucleotide encoding such polypeptide.

FIG. 13 B shows one embodiment of the OMP-1U protein, FIG 13A shows one embodiment of the OMP-1U polynucleotide.

FIG. 14 B shows one embodiment of the OMP-1V protein, FIG 14A shows one embodiment of the OMP-1V polynucleotide.

FIG. 15 B shows one embodiment of the OMP-1W protein, FIG 15A shows one embodiment of the OMP-1W polynucleotide.

FIG. 16 B shows one embodiment of the OMP-1X protein, FIG 16A shows one embodiment of the OMP-1W polynucleotide.

FIG. 17 B shows one embodiment of the OMP-1Y protein, FIG 17A shows one embodiment of the OMP-1Y polynucleotide.

FIG. 18 B shows one embodiment of the OMP-1Z protein, FIG 18A shows one embodiment of the OMP-1Z polynucleotide.

FIG. 19 B shows one embodiment of the P30 protein, FIG 19A shows one embodiment of the P30 polynucleotide.

FIG. 20 B shows one embodiment of the P30a protein, FIG 20A shows one embodiment of the p30A polynucleotide.

FIG. 21 B shows one embodiment of the P30-1 protein, FIG 21A shows one embodiment of the p30-1 polynucleotide.

FIG. 22 B shows one embodiment of the P30-2 protein, FIG 22 A shows one embodiment of the p30-2 polynucleotide.

FIG. 23 B shows one embodiment of the P30-3 protein, FIG 23 A shows one embodiment of the p30-3 polynucleotide.

FIG. 24 B shows one embodiment of the P30-4 protein, FIG 22 A shows one embodiment of the p30-4 polynucleotide.

FIG. 25 B shows one embodiment of the P30-5 protein, FIG 22 A shows one embodiment of the p30-5 polynucleotide.

FIG. 26 B shows one embodiment of the P30-6 protein, FIG 26 A shows one embodiment of the p30-6 polynucleotide.

FIG. 27 B shows one embodiment of the P30-7 protein, FIG 27 A shows one embodiment of the p30-7 polynucleotide.

FIG. 28 B shows one embodiment of the P30-8 protein, FIG 28 A shows one embodiment of the p30-8 polynucleotide.

FIG. 29 B shows one embodiment of a portion of the P30-9 protein, FIG 29 A shows one embodiment of the p30-9 polynucleotide encoding such polypeptide.

FIG. 30 B shows one embodiment of a portion of the P30-10 protein, FIG 30 A shows one embodiment of the p30-10 polynucleotide encoding such polypeptide.

FIG. 31 depicts the amino acid sequences alignment of seven *E. chafeensis* OMP-1s and *Cowdria ruminantium* MAP-1. Aligned positions of identical amino acids with OMP-1F are shown with dots. The sequence of *C. ruminantium* MAP-1 is from the report of Van Vliet et al (1994) Molecular cloning, sequence analysis, and expression of the gene encoding the immunodominant 32-kilodalton protein of *Cowdria ruminantium*. Infect. Immun. 62:1451-1456. Gaps indicated by dashes were introduced for optimal alignment of all proteins. Bars indicates semivariable region (SV) and three hypervariable regions (HV1, HV2, and HV3).

DETAILED DESCRIPTION OF THE INVENTION

Isolated Polynucleotides Encoding OMP-1,OMP-1A, OMP-1B, OMP-1C, OMP-1D, OMP-1F and the OMP from E. Canis

In one aspect, the present invention, provides isolated polynucleotides that encode the outer membrane proteins, OMP-1 (or p28), OMP-1B, OMP-1C, OMP-1D, OMP- 1E, OMP-1F, OMP-1A, OMP-1R, OMP-1S, OMP-1T, OMP -1U, OMP-1V, OMP-1W, OMP-1X, OMP-1Y and OMP-1Z from E. chafeensis and the outer membrane proteins P30, P30-a, P-30-1, P30-2, P30-3, P30-4, P30-5, P30-6, P30-7, P30-8, P30-9, and P30-10 from E. Canis or an immunogenic fragment thereof.

The polynucleotide is single stranded or double stranded. The polynucleotide may be a DNA or RNA molecule, preferably a DNA molecule, and comprises a sequence which codes for the respective outer membrane protein. Preferably, the polynucleotide encodes at least the mature form of outer membrane protein. The polynucleotide optionally further comprises a leader sequence and encode an outer membrane preprotein that is

processed in the cell to form the mature protein. The polynucleotide of the present invention may also be fused in frame to a marker sequence which allows for purification of the corresponding outer membrane protein.

The OMP-1 polynucleotide encodes an OMP-1 protein of E. chafeensis having a molecular weight of about 27.7 kDa and an amino acid sequence which is at least 85% homologous to the amino acid sequence shown in FIG. 3B SEQ ID NO: __; Figure 3B shows one embodiment of the OMP-1 protein, Figure 3A shows one embodiment of the OMP-1 polynucleotide. The OMP-1B polynucleotide encodes an OMP-1B protein of E. chafeensis having a molecular weight of about 28.2 kDa and an amino acid sequence which is at least 85% homologous to the amino acid sequence shown in FIG. 4B SEQ ID NO: __; Figure 4B shows one embodiment of the OMP-1B protein, Figure 4A shows one embodiment of the OMP-1B polynucleotide. The OMP-1C polynucleotide encodes an OMP-1C protein of E. chafeensis having a molecular weight of about 27.6 kDa and an amino acid sequence which is at least 85% homologous to the amino acid sequence shown in FIG. 5B SEQ ID NO: __; Figure 5B shows one embodiment of the OMP-1C protein, Figure 5A shows one embodiment of the OMP-1C polynucleotide. The OMP-1D polynucleotide encodes an OMP-1D protein of E. chafeensis having a molecular weight of about 28.7 kDa and an amino acid sequence which is at least 85% homologous to the amino acid sequence shown in FIG. 6B SEQ ID NO: __; Figure 6B shows one embodiment of the OMP-1D protein, Figure 6A shows one embodiment of the OMP-1D polynucleotide. The OMP-1E polynucleotide encodes an OMP-1E protein of E. chafeensis having a molecular weight of about 27.8 kDa and an amino acid sequence which is at least 85% homologous to the amino acid sequence shown in FIG. 7B SEQ ID NO: __; Figure 7B shows one embodiment of the OMP-1E protein, Figure 7A shows one embodiment of the OMP-1E polynucleotide. The OMP-1F polynucleotide encodes an OMP-1F protein of E. chafeensis having a molecular weight of about 27.9 kDa and an amino acid sequence which is at least 85% homologous to the amino acid sequence shown in FIG. 8B SEQ ID NO: __; Figure 8B shows one embodiment of the OMP-1F protein, Figure 8A shows one embodiment of the OMP-1F polynucleotide. The OMP-1A polynucleotide encodes an OMP-1A protein of E. chafeensis having a molecular weight of about 29.6 kDa and an amino acid sequence which is at least 85% homologous to the amino acid sequence shown in FIG. 9B SEQ ID NO: __; Figure 9B shows one embodiment of the OMP-1A protein, Figure 9A shows one embodiment of the OMP-1A polynucleotide. The OMP-1R polynucleotide encodes an OMP-1R protein of E. chafeensis having a molecular weight of at least 23 kDa and comprising an amino acid sequence which is at least 85% homologous to the amino acid sequence shown in FIG. 10B SEQ ID NO: __; Figure 10B shows one embodiment of a portion of the OMP-1R protein, Figure10A shows one embodiment of the OMP-1R polynucleotide encoding such polynucleotide. The OMP-1S polynucleotide encodes an OMP-1S protein of E. chafeensis comprising an amino acid sequence which is at least 85% homologous to the amino acid sequence shown in FIG. 11B SEQ ID NO: __; Figure 11B shows one embodiment of a portion of the OMP-1S protein, Figure 11A shows one embodiment of the OMP-1S polynucleotide encoding such polypeptide. The OMP-1T polynucleotide encodes an OMP-1T protein of E. chafeensis comprising an amino acid sequence which is at least 85% homologous to the amino acid sequence shown in FIG.12B SEQ ID NO: __; Figure 12B shows one embodiment of a portion of the OMP-1T protein, Figure 12B shows one embodiment of a polynucleotide encoding such polypeptide. The OMP-1U polynucleotide encodes an

OMP-1U protein of E. chafeensis having a molecular weight of about 30.6 kDa and an amino acid sequence which is at least 85% homologous to amino acid sequence shown in FIG. 13B SEQ ID NO: __; Figure 13B shows one embodiment of the OMP-1U protein, Figure 13A shows one embodiment of the OMP-1U polynucleotide. The OMP-1V polynucleotide encodes an OMP-1V protein of E. chafeensis having a molecular weight of about 28.0 kDa and an amino acid sequence which is at least 85% homologous to the amino acid sequence shown in FIG. 14B SEQ ID NO: __; Figure 14B shows one embodiment of the OMP-1V protein, Figure 14A shows one embodiment of the OMP-1V polynucleotide. The OMP-1W polynucleotide encodes an OMP-1W protein of E. chafeensis having a molecular weight of about 28.8 kDa and an amino acid sequence which is at least 85% homologous to the amino acid sequence shown in FIG. 15B SEQ ID NO: __; Figure 15B shows one embodiment of the OMP-1W protein, Figure 15A shows one embodiment of the OMP-1W polynucleotide. The OMP-1X polynucleotide encodes an OMP-1S protein of E. chafeensis having a molecular weight of about 27.8 kDa and an amino acid sequence which is at least 85% homologous to the amino acid sequence shown in FIG. 16B SEQ ID NO: __; Figure 16B shows one embodiment of the OMP-1X protein, Figure 16A shows one embodiment of the OMP-1X polynucleotide. The OMP-1Y polynucleotide encodes an OMP-1Y protein of E. chafeensis having a molecular weight about 28.8 kDa and an amino acid sequence which is at least 85% homologous to the amino acid sequence shown in FIG. 17B SEQ ID NO: __; Figure 17B shows one embodiment of the OMP-1Y protein, Figure 17A shows one embodiment of the OMP-1Y polynucleotide. The OMP-1Z polynucleotide encodes an OMP-1Z protein of E. chafeensis comprising an amino acid sequence which is at least 85% homologous to the amino acid sequence shown in FIG. 18B SEQ ID NO: __; Figure 18B shows one embodiment of a portion of the OMP-1Z protein, Figure 18A shows one embodiment of an OMP-1Z polynucleotide encoding such polypeptide.

The p30 polynucleotide encodes a P30 protein of E. canis having a molecular weight of about 28.8 kDa and an amino acid sequence which is at least 85% homologous to the amino acid sequence shown in FIG. 19B SEQ ID NO: __; Figure 19B shows one embodiment of the P30 protein, Figure 19A shows one embodiment of the p30 polynucleotide. The p30A polynucleotide encodes a P30a protein of E. canis having a molecular weight of about 29.1 kDa and an amino acid sequence which is at least 85% homologous to the amino acid sequence shown in FIG. 20B SEQ ID NO: __; Figure 20B shows one embodiment of the P30a protein, Figure 20A shows one embodiment of the p30A polynucleotide. The p30-1 polynucleotide encodes a P30-1 protein of E. canis having a molecular weight of about 27.7 kDa and an amino acid sequence which is at least 85% homologous to the amino acid sequence shown in FIG. 21B SEQ ID NO: __; Figure 21B shows one embodiment of the P30-1 protein, Figure 21A shows one embodiment of the p30-1 polynucleotide. The p30-2 polynucleotide encodes a P30-2 protein of E. canis having a molecular weight of about 28.0 kDa and an amino acid sequence which is at least 85% homologous to the amino acid sequence shown in FIG. 22B SEQ ID NO: __; Figure 22B shows one embodiment of the P30-2 protein, Figure 22A shows one embodiment of the p30-2 polynucleotide. The p30-3 polynucleotide encodes a P30-3 protein of E. canis having a molecular weight of about 28.7 kDa and an amino acid sequence which is at least 85% homologous to the amino acid sequence shown in FIG. 23B SEQ ID NO: __; Figure 23B shows one embodiment of the P30-3 protein, Figure 23A shows one embodiment of the p30-3 polynucleotide. The p30-4 polynucleotide

encodes a P30-4 protein of E. canis having a molecular weight of about 28.0 kDa and an amino acid sequence which is at least 85% homologous to the amino acid sequence shown in FIG. 24B SEQ ID NO: __; Figure 24B shows one embodiment of the P30-4 protein, Figure 24A shows one embodiment of the p30-4 polynucleotide. The p30-5 polynucleotide encodes a P30-5 protein of E. canis having a molecular weight of about 29.4 kDa and an amino acid sequence which is at least 85% homologous to the amino acid sequence shown in FIG. 25B SEQ ID NO: __; Figure 25B shows one embodiment of the P30-5a protein, Figure 25A shows one embodiment of the p30-5a polynucleotide. The p30-6 polynucleotide encodes a P30-6 protein of E. canis having a molecular weight of about 29.5 kDa and an amino acid sequence which is at least 85% homologous to the amino acid sequence shown in FIG. 26B SEQ ID NO: __; Figure 26B shows one embodiment of the P30-6 protein, Figure 26A shows one embodiment of the p30-6 polynucleotide. The p30-7 polynucleotide encodes a P30-7 protein of E. canis having a molecular weight of about 29.9 kDa and an amino acid sequence which is at least 85% homologous to the amino acid sequence shown in FIG. 29B SEQ ID NO: __; Figure 29B shows one embodiment of the P30-7 protein, Figure 29A shows one embodiment of the p30-7 polynucleotide. The p30-8 polynucleotide encodes a P30-8 protein of E. canis having a molecular weight of about 30.3 kDa and an amino acid sequence which is at least 85% homologous to the amino acid sequence shown in FIG. 28B SEQ ID NO: __; Figure 28B shows one embodiment of the P30-8 protein, Figure 28A shows one embodiment of the p30-8 polynucleotide. The p30-9 polynucleotide encodes a P30-9 protein of E. canis comprising an amino acid sequence which is at least 85% homologous to the amino acid sequence shown in FIG. 29B SEQ ID NO: __; Figure 29B shows one embodiment of a portion of the P30-9 protein, Figure 29A shows one embodiment of the p30-9 polynucleotide encoding such polypeptide. The p30-10 polynucleotide encodes a P30-10 protein of E. canis comprising an amino acid sequence which is at least 85% homologous to the amino acid sequence shown in FIG. 30B SEQ ID NO: __; Figure 30B shows one embodiment of a portion of the P30-10 protein, Figure 30A shows one embodiment of the p30-10 polynucleotide encoding such polypeptide.

The polynucleotides encoding an E. chafeensis outer membrane protein or an E. canis outer membrane protein have a sequence that is at least 85%, preferably at least 90%, more preferably at least 95% homologous to or similar to the amino acid sequences shown in Figures 3B through 30B, and thus embrace polynucleotides encoding outer membrane proteins from different strains of E. chafeensis and E. canis. The polynucleotides encode an outer membrane protein whose conserved regions collectively are at least 90%, preferably at 95%, more preferably at least 97% homologous to the conserved regions of the amino acid sequences of the present invention. The outer membrane proteins of E. chafeensis and E. canis have six conserved regions, which are separated by one semivariable region and three hypervariable regions. The conserved regions of the outer membrane proteins OMP-1, OMP-1A, OMP-1B, OMP1-C, OMP-1D, OMP1-F are depicted in Fig. 31. Preferably, the amino acid sequence of the outer membrane proteins of E. chafeensis and E. canis are at least 30% divergent from the amino acid sequence of MAP-1. Such sequences include allelic, strain variants and other amino acid sequence variants (e.g., including "muteins" or "mutant proteins"), whether naturally-occurring or biosynthetically produced. As used herein, "amino acid sequence homology" is understood to mean amino acid sequence similarity, and homologous sequences share identical or similar amino acids, where similar amino acids are conserved amino acids as defined by

Dayoff et al., *Atlas of Protein Sequence and Structure*; vol. 5, Supp. 3, pp. 345-362 (M. O. Dayoff, ed., Nat'l BioMed. Research Fdn., Washington D.C. 1978.) Thus, a candidate sequence sharing 85% amino acid sequence homology with a reference sequence requires that, following alignment of the candidate sequence with the reference sequence, 85% of the amino acids in the candidate sequence are identical to the corresponding amino acid in the reference sequence, or constitute a conserved amino acid change thereto. "Amino acid sequence identity" is understood to require identical amino acids between two aligned sequences. Thus, a candidate sequence sharing 85% amino acid identity with a reference sequence requires that, following alignment of the candidate sequence with the reference sequence, 85% of the amino acids in the candidate sequence are identical to the corresponding amino acid in the reference sequence.

As used herein, all homologies and identities are calculated using the amino acid sequences shown in the cited Figure or SEQ ID NO as the reference sequence. Thus, to determine whether an amino acid sequence is 85% homologous to OMP-1, one uses the amino acid sequence shown in Fig. ___, SEQ ID NO: ___ as a reference.

Also as used herein, sequences are aligned for homology and identity calculations using the method of the software basic local alignment search tool in the BLAST network service (the National Center for Biotechnology Information, Bethesda, MD) which employs the method of Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990) *J. Mol. Biol.* 215, 403-410. Identities are calculated by the Align program (DNAstar, Inc.) In all cases, internal gaps and amino acid insertions in the candidate sequence as aligned are ignored when making the homology/identity calculation.

In another aspect, the present invention provides a nucleotide sequence encoding a polypeptide which comprises a fragment of the OMP1 protein, hereinafter referred to as "rP28" . The rP28 polypeptide weighs approximately 31 kDa and comprises all but of the first 5 amino acids of mature OMP-1 protein. The rP28 polypeptide comprises the amino acid sequence extending from amino acid 6 through amino acid 251 of the amino acid sequence shown in Fig.1, SEQ ID NO. The present invention also embraces polypeptides where one or more of the amino acids in the sequence extending from amino acid 1 or 6 through amino acid 251 Fig. 1 are replaced by conservative amino acid residues. The present invention also relates to derivatives of rP28 that have an amino acid sequence identity of at least 85%, more preferably at least 90%, and most preferably of at least 95% with the amino acid sequence extending from amino acid 1 or 6 through amino acid 251 of the protein and which derivative binds to antibodies in sera from humans infected with E. chafeensis.

The polynucleotides are useful for producing the outer membrane proteins of E. chafeensis and E. canis. For example, an RNA molecule encoding the outer membrane protein OMP-1is used in a cell-free translation systems to prepare OMP-1. Alternatively, a DNA molecule encoding the outer membrane protein is introduced into an expression vector and used to transform cells. Suitable expression vectors include for example chromosomal, nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40, bacterial plasmids, phage DNAs; yeast plasmids, vectors derived from combinations of plasmids and phage DNAs, viral DNA such as vaccinia, adenovirus, fowl pox virus, and pseudorabies. The DNA sequence is introduced into the expression vector by conventional procedures.

Accordingly, the present invention also relates to recombinant constructs comprising one or more of the polynucleotide sequences. Suitable constructs include, for example, vectors, such as a plasmid, phagemid, or viral vector, into which a sequence that encodes the outer membrane protein has been inserted. In the expression vector, the DNA sequence which encodes the outer membrane protein is operatively linked to an expression control sequence, i.e., a promoter, which directs mRNA synthesis. Representative examples of such promoters, include the LTR or SV40 promoter, the *E .coli* lac or trp, the phage lambda PL promoter and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or in viruses. The promoter may also be the natural promoter of the outer membrane protein coding sequence. The expression vector also contains a ribosome binding site for translation initiation and a transcription terminator. Preferably, the recombinant expression vectors also include an origin of replication and a selectable marker, such as for example, the ampicillin resistance gene of *E. coli* to permit selection of transformed cells, i.e. cells that are expressing the heterologous DNA sequences. The polynucleotide sequence encoding the outer membrane protein is incorporated into the vector in frame with translation initiation and termination sequences. Optionally, the sequence encodes a fusion outer membrane protein which includes an N-terminal or C-terminal peptide or tag that stabilizes or simplifies purification of the expressed recombinant product. Representative examples of such tags include sequences which encode a series of histidine residues, the Herpes simplex glycoprotein D, or glutathione S-transferase.

Polynucleotides which encode portions of the outer membrane proteins of E. chafeensis and E. canus are useful as probes for isolating and identifying E. chafeensis genes and E. canis genes, particularly full-length genes from new strains or isolates of E. chafeensis and E. canis.

The Outer Membrane Proteins of E. chafeensis and E. Canis

In addition to the outer membrane proteins OMP-1, OMP-1B, OMP-1C, OMP-1D, OMP-1 E, and OMP-1F, OMP-1R, OMP-1S, OMP-1T, OMP-1U, OMP-1V, OMP-1W, OMP-1X, OMP-1Y, and OMP-1Z from E. chafeensis and the proteins P30, P30A, P30-1, P30-2, P30-3, P30-4, P30-5, P30-6, P30-7, P30-8, P30-9, and P30-10 from E. Canis, the present inventions embraces non-naturally occurring allelelic forms or derivatives of the outer membrane proteins, where one or more of the amino acids have been replaced by conservative amino acid residues, typically by using direct synthesis or recombinant techniques.

Preparing the Outer Membrane Proteins

The outer membrane proteins of the present invention are synthetically produced by conventional peptide synthesizers. The outer membrane proteins are also produced using cell-free translation systems and RNA molecules derived from DNA constructs that encode the outer membrane protein. Alternatively, the outer membrane protein is made by transfecting host cells with expression vectors that comprise a DNA sequence which encodes the outer membrane protein and then inducing expression of the outer membrane protein in the host cells.

The outer membrane protein is expressed in suitable host cells, preferably bacteria, under the control of suitable promoters. Host cells are transformed with the expression vectors of this invention and cultured in conventional nutrient media. Such media optionally contains additional compounds, such as for example

compounds that induce promoters, such as for example isopropyl-β-D-thiogalactoside which induces the Lac promoter, or compounds, such as for example, ampicillin, which allows for selection of transformants.

Following transformation of the suitable host strain and growth of the host strain to an appropriate cell density, the cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification of the outer membrane protein. Such purification usually involves salting-out of the protein fraction, and one or more chromatography steps, including aqueous ion exchange chromatography, size exclusion chromatography steps, and high performance liquid chromatography (HPLC).

Preparation of Antibodies .

The isolated outer membrane proteins, particularly the recombinant forms of the outer membrane proteins, are used as immunogens to produce antibodies immunospecific for the corresponding protein. The term "immunospecific" means the antibodies have substantially greater affinity for the protein used as an immunogen than for other proteins. Such antibodies are generated using conventional techniques by administering the respective outer membrane protein or a portion thereof, i.e., the recombinant polypeptide, to an animal, preferably a nonhuman. collecting blood from the immunized animals and isolating the serum and/or the IgG fraction from the blood. Monoclonal antibodies are prepared by injecting animals with the immunogens, extracting antibody-producing B cells from the animal, fusing the B cells with a myeloma cells to produce hybridomas, obtaining the monoclonal antibodies from the hybridomas.

Antibodies to the outer membrane proteins of E. chafeensis and E. canis are useful research tools for identifying cells, particularly monocytes, infected with E.chafeensis or E. canis and for purifying the corresponding outer membrane protein of E.chafeensis or E. Canis from partially purified preparations by affinity chromatography. Such antibodies are also useful for identifying bacterial colonies, particularly colonies of genetically-engineered bacteria, that are expressing the major outer membrane protein .

Diagnostic Method

The present invention also provides a method for detecting antibodies to the E. chafeensis or E.canis in a sample of a bodily fluid from a patient. The method comprises providing an isolated outer membrane protein of E. chafeensis or E. canis, particularly a recombinant form of the isolated protein, contacting the outer membrane protein or polypeptide with a sample taken from the patient; and assaying for the formation of a complex between the outer membrane protein or polypeptide and antibodies in the sample. For ease of detection, it is preferred that the isolated protein or polypeptide be attached to a substrate such as a column, plastic dish, matrix, or membrane, preferably nitrocellulose. The sample may be a tissue or a biological fluid, including urine, whole blood, or exudate, preferably serum. The sample may be untreated, subjected to precipitation, fractionation, separation, or purification before combining with the isolated protein or peptide. Interactions between antibodies in the sample and the isolated protein or peptide are detected by radiometric, colorimetric, or fluorometric means, size-separation, or precipitation. Preferably, detection of the antibody-outer membrane protein complex is by addition of a secondary antibody that is coupled to a detectable tag, such as for example, an enzyme, fluorophore, or chromophare. Formation of the complex is indicative of the presence of anti-E chafeensis or anti-E canis antibodies,

either IgM or IgG, in the patient. Thus, the method is used to determine whether a patient is infected with E. chafeensis or E. canis.

Preferably, the method employs an enzyme-linked immunosorbent assay (ELISA) or a Western immunoblot procedure . Such methods are relatively simple to perform and do not require special equipment as long as membrane strips are coated with a high quality antigen. Accordingly, it is more advantageous to use a recombinant form of the outer membrane protein of E. chafeensis or E. canis since such proteins, typically, are more pure and consistent in quality than a purified form of such protein.

Immunogenic Composition

The present invention also relates to immunogenic compositions comprising one or more of the isolated outer membrane proteins of E. chafeensis and a pharmaceutically acceptable adjuvant and to immunogenic compositions comprising an isolated P30 protein of E. canis and a pharmaceutically acceptable adjuvant, which, preferably, enhances the immunogenic activity of the outer membrane protein in the host animal.

Preparation of a Polynucleotide which Encodes OMP-1(P28)

A. Isolation of the Outer Membrane Proteins

E. chafeensis Arkansas strain and *E. canis* Oklahoma strain were cultivated in the DH82 dog macrophage cell line and purified by Percoll density gradient centrifugation. Purified ehrlichiae (100 µg) were suspended with 10 mM sodium phosphate buffer, pH 7.4, containing 0.1% Sodium *N*-lauroyl sarcosine (Sarkosyl) [Sigma, St. Louis, MO], 50 µg/ml each Dnase I (Sigma) and Rnase A (Sigma), and 2.5 mM $MgCl_2$. After incubation at 37° for 30 min, the sample was separated by centrifugation at 10,000 x g for 1 h into the soluble supernatant and the insoluble precipitate. The insoluble pellet was resuspended 2 to 3 times with 0.1% Sarkosyl and centrifuged. The final pellet was analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and by electron microscopy.

Transmission electron microscopy revealed that the purified ehrlichial fraction consists of a mixture of electron dense and light forms of *E. chafeensis* with slight disintegration of inner membrane . Ehrlichiae were not surrounded with the host inclusion membrane. Various sizes of membrane vesicles (< 1 µm) without significant ribosomes or nuclear materials were observed in the Sarkosyl-insoluble fraction from the organism. Succinic dehydrogenase (inner membrane marker enzyme of gram negative bacteria) activities were at less than the detection limit (1 n moles / min / mg of protein) in the Sarkosyl-insoluble fraction compared to approximately 10 n moles / min / mg of protein in the Percoll-purified organisms, suggesting that the insoluble fraction primarily consisted of the outer membrane of *E. chafeensis*.

Analysis of the Sarkosyl-soluble, and insoluble fraction of *E. chafeensis* by SDS-PAGE suggested that proteins of 30-kDa range in the insoluble fraction represent the major outer membrane proteins of this organism. Analysis of the Sarkosyl-soluble, and insoluble fraction of *E. canis* by SDS-PAGE suggested that proteins of 30-kDa range in the insoluble fraction represent the major outer membrane proteins of this organism also. *E. canis* was

antigenically cross reactive with *E. chafeensis*. These findings indicate that the 30-kDa range proteins represent the major outer membrane proteins of these two *Ehrlichia* spp.

To improve resolution of the outer membrane proteins, proteins in the Sarkosyl-insoluble pellet prepared from 400 µg of purified *E. chafeensis* were separated by a reversed-discontinuous (Rd) SDS-PAGE (2.5-cm-long 17% gel on top of 11-cm-long 12% gel). At least five proteins of 30-kDa range in *E. chafeensis* (P23, P25, P27, P28, and P29) were resolved from the Sarkosyl-insoluble proteins.

B. Cloning and sequencing of the *p28* gene

The portion of the membrane containing bound proteins was excised and analyzed with an Applied Biosystems protein sequencer (Model 470). The N-terminal amino acid sequence of P28 was determined as D P A G S G I N G N F Y S G K Y M P, SEQ IN NO _____. Based on 6th to 12th amino acids of this sequence, a forward primer, FECH1, having the sequence: 5'-CGGGATCCGAATTCGG(A/T/G/C)AT(A/T/C)AA(T/C)GG(A/T/G/C)AA(T/C)TT(T/C)TA-3'. SEQ ID NO _____ was designed. Amino acids at the 1 to 5 positions of the N terminus of P28 were not included in this primer design. For insertion into an expression vector, a 14-bp sequence (underlined) was added at the 5' end of primer to create an *Eco*RI and a *Bam*HI site. The reverse primer, RECH2, which includes a *Not*I site at the 5' end for ligation into an expression vector had the sequence : 5'-AGCGGCCGCTTA(A/G)AA(T/C)A(C/G) (A/G)AA (C/T)CT T(C/G)C TCC-3'. SEQ ID NO _____.

Genomic DNA of *E. chafeensis* was isolated from purified organisms. PCR amplification with FECH1 and RECH2 primers was performed using a Perkin-Elmer Cetus DNA Thermal Cycler (model 480). A 0.8-kb amplified product was cloned in the pCRII vector of a TA closing kit, as described by the manufacturer (Invitrogen Co., San Diego, CA). The clone obtained was designated pCRII*p28*. Both strands of the inserted DNA were sequenced by a dideoxy-termination method with an Applied Biosystems 373A DNA sequencer.

The 0.8-kb DNA fragment, cloned in pCRII*p28*, had an open reading frame (ORF) of 756 bp encoding a 251-amino acid recombinant protein (including both PCR primer regions) with a molecular mass of 27,685 Da. The nucleotide sequence of the open reading frame, SEQ ID NO: , and the amino acid sequence of the polypeptide of the OMP-1 protein, SEQ ID NO ___, are shown in Figs _____ and _____, respectively.

A DNA fragment comprising the *p30* gene was prepared in a similar manner, i.e., by PCR amplification of genomic DNA of E. canis with the FECH1 and RECH2 primers.

Preparation of Polynucleotides which encode OMP 1A, OMP1B, OMP1-C, OMP-1D, OMP-1F, and OMP1-E

A. Southern blot analysis. Genomic DNA extracted from the purified *E. chafeensis* (200 ng each) was digested with restriction endonucleases, electrophoresed, and transferred to Hybond-N$^+$ nylon membrane (Amersham, Arlington Heights, IL), by a standard method. The 0.8-kb *p28* gene fragment from the clone pCRII*p28* was labeled with [α-^{32}P]dATP by the random primer method using a kit (Boehringer Mannheim, Indianapolis, IN) and the labeled fragment was used as a DNA probe. Hybridization was performed at 60°C in rapid hybridization buffer (Amersham) for 20 h. The nylon sheet was washed in 0.1 x SSC (1 x SSC containing 0.15M sodium chloride and

0.015M sodium citrate) with 1% SDS at 55°C and the hybridized probes were exposed to Hyperfilm (Amersham) at -80°C.

Genomic Southern blot analysis with several restriction enzymes resulted in one or more DNA fragment(s) of *E. chafeensis* which hybridized to ^{32}P-labeled *p28* gene probe. The restriction enzymes used did not cut within the *p28* gene portion of the pCRII*p28* insert. *Xba* I, *Bgl* II, and *Kpn* I produced two bands, *Spe* I generated three bands, and *Eco*R V and *Pst* I produced multiple bands with different densities. *Eco*R I generated a broad band of 2.5 to 4kb. These *p28* homologous genes are designated as *omp-1* (outer membrane protein-1) family.

B. Cloning and sequencing of genomic copies of *E. chafeensis p28* gene. The *EcoR* I and *Pst* I fragments of DNA, detected by genomic Southern blot analysis as described above, were inserted into pBluescript II KS (+) vectors, and the recombinant plasmids were introduced into *E. coli* DH5α. Using the colony hybridization method with the ^{32}P-labeled *p28* gene probe, four positive clones were isolated from the transformant. The positive clones were designated pEC2.6, pEC3.6, pPS2.6, and pPS3.6. These contained the ehrlichial DNA fragments of 2.6-kb (*Eco*R I), 3.6 kb (*Eco*R I), 2.6 kb (*Pst* I), and 3.6 kb (*Pst* I), respectively. The inserts of the clones pEC3.6 and pPS2.6 overlapped as shown in Fig.____. The overlapping area was further confirmed by PCR of *E. chafeensis* genomic DNA with two pairs of primer sets interposing the junctions of the four clones. The 1.1- to 1.6-kb DNA fragments of *Hin*dIII-*Hin*dIII, *Hin*dIII-*Eco*RI, or *Xho*I-*Eco*RI in the pEC2.6 and pEC3.6 were subcloned for sequencing. DNA sequencing was performed with suitable synthetic primers by dideoxy-termination method as described above.

Four DNA fragments from 2.6 to 3.6 kb were cloned from the *Eco*RI-digested and the *Pst*I-digested genomic DNA of *E. chafeensis* by colony hybridization with radiolabeled *p28* gene probe. The inserted DNA of the two recombinant clones, pEC3.6 and PPS2.6, were overlapped as shown in Fig. 7. Sequencing revealed one 5'-truncated ORF of 243 bp (designated *omp-1*A) and five complete ORF of 836-861 bp (designated *omp-1*B to *omp-1*F), which are tandemly-arrayed and are homologous to the *p28* gene (but are not identical), in the ehrlichial genomic DNA of 6,292 bp. The intergenic spaces were 581 bp between *omp-1*A and *omp-1*B and 260-308 bp among others. Putative promoter regions and ribosome-binding sites were identified in the noncoding regions.

Sequence analysis and GenBank accession number.

Nucleotide sequences were analyzed with the DNASIS program (Hitachi Software Engineering Co., Ltd., Yokohama, Japan). A homology search was carried out with databases of the GenBank, Swiss Plot, PDB and PIR by using the software basic local alignment search tool in the BLAST network service (the National Center for Biotechnology Information, Bethesda, MD). Phylogenetic analysis was performed by using the PHYLIP software package (version 3.5). An evolutional distance matrix, generated by using the Kimura formula in the PROTDIST, was used for construction of a phylogenetic tree by using the unweighted pair-group method analysis (UPGMA) (Felsenstein, J. 1989. PHYLIP-phylogeny inference package (version 3.3). Cladistics 5:164-166). The data were also examined using parsimony analysis (PROTPARS in PHYLIP). A bootstrap analysis was carried out to investigate the stability of randomly generated trees by using SEQBOOT and CONSENSE in the same package. The nucleotide sequence of the *p28* gene and its gene copies has been assigned GenBank accession numbers U72291 and AF021338, respectively.

Proteins of the *E. chafeensis omp-1* Family.

Five complete *omp-1* gene copies (*omp-1B* to *omp-1*F) encode 279 to 287-amino acid proteins with molecular masses of 30,320 - 31,508 Da. *Omp-1A* encodes an 82-amino acid partial protein (9,243 Da) which lacks the N-terminal region. The 25-amino acid sequence at the N-terminus of OMP-1B to OMP-1F (encoded in *omp-1*B to *omp-1*F) is predicted to be a signal peptide because three carboxyl-terminal amino acids of the signal peptides (Ser-X-Ala in OMP-1B, Leu-X-Ser for OMP-C, and Ser-X-Ser for OMP-1D and OMP-1F) are included in the preferred amino acid sequence of signal peptidase at the processing sites proposed by Oliver .. The calculated molecular masses of the mature OMP-1B to OMP-1F from the predicted amino acid sequences are 28,181 Da for OMP-1B, 27,581 Da for OMP-1C, 28,747 Da for OMP-1D, 27,776 Da for OMP-1E, and 27,933 Da for OMP-1F. The estimated isoelectric points are 4.76-5.76 in the mature OMP-1B to OMP-1F. An amino acid sequence in *omp-1F* gene (the 80th to 94th amino acids) was identical to the N-terminal amino acid sequences of *E. chafeensis* native P23 protein as determined chemically, which indicates that P23 is derived from the *omp-1F* gene. Amino acid sequences identical to the N-terminal sequences of P25, P27, and P29 were not found in those from *omp-1* gene copies cloned in this study.

Alignment of predicted amino acid sequences of the *E. chafeensis* OMP-1 family and *Cowdria ruminantium*, revealed substitutions or deletions of one or several contiguous amino acid residues throughout the molecules. The significant differences in sequences among the aligned proteins are seen in the regions indicated SV (semivariable region) and HV (hypervariable region) 1 to 3 in Fig 3I. Computer analysis for hydropathy revealed that protein molecules predicted from all *omp-1* gene copies contain alternative hydrophilic and hydrophobic motifs which are characteristic of transmembrane proteins. The HV1 and HV2 were found to locate in the hydrophilic regions.

The amino acid sequences of 5 mature proteins without signal peptides (OMP-1C to OMP-1F and a P28) were similar to one another (71-83%) but the sequence of OMP-1B was dissimilar to those of the 5 proteins (45-48%). The amino acid sequences of the 5 proteins showed an intermediate degree of similarity with that of *C. ruminantium* MAP-1 (59-63%), but the similarity between that of the OMP-1B and the *C. ruminantium* MAP-1 was low (45%). These relations are shown in a phylogenetic tree which was obtained based on the amino acid sequence alignment by UPGMA method in the PHYLIP software package (Fig. 10). Three proteins (P28, OMP-1D, and OMP-1F) and two proteins (OMP-1C and OMP-1E) formed two separate clusters. The OMP-1B was located distantly from these two clusters. The *C. ruminantium* MAP-1 was positioned between the OMP-1B and other members in the OMP-1 family.

Preparation of a Recombinant form of OMP-1 and P30

The 0.8-kb *p28* gene was excised from the clone pCRII*p28* by *Eco*RI-*Not*I double-digestion, ligated into *Eco*RI-*Not*I sites of a pET 29a expression vector, and amplified in *Escherichia coli* BL21 (DE3)pLysS (Novagen, Inc., Madison, WI). The clone (designated pET29*p28*) produced a fusion protein with a 35-amino acid sequence

carried from the vector at the N terminus. The amino acid sequence of the OMP-1 portion of the fusion protein is depicted in Fig. 1.

An expression vector comprising the p30 gene was used to prepare the recombinant form of P30.

The following examples are for purposes of illustration only and are not intended to limit the scope of the claims which are appended hereto.

Preparation of anti rP28 (anti-OMP1) antibody

The (r) P28 antigen was prepared by excising the gel band corresponding to the rP28 in SDS-PAGE, mincing the band in phosphate-buffered saline (PBS), *p*H 7.4, and mixing with an equal volume of Freund's incomplete adjuvant (Sigma). The rP28 mixture (1 mg of protein each time) was subcutaneously injected into a rabbit every 2 weeks four times. A serum sample was collected from the rabbit to provide the anti-rP28 antibody

The anti-rP28 antibody was examined by western immunoblots analysis. The results indicated that the rabbit anti-rP28 antibody recognized not only rP28 (31 kDa) and P28, but also P29 and P25 of *E. chafeensis* and P30 of *E. canis* . These results indicate that P28 shares antigenic epitopes with P25 and P29 in *E. chafeensis* and P30 of *E. canis*.

Example 1. Assaying for the presence of anti-OMP-1 antibody in a Patient

Convalescent-phase serum from a patient with clinical signs of human ehrlichiosis was used. Western blot analyses using the rP28 protein as antigen was performed with 1:1,000 dilutions of this serum. Alkaline phosphatase-conjugated affinity-purified anti-human, anti-rabbit or anti-mouse immunoglobulin G (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD) were used at a 1:1,000 or 1:2,000 dilution as secondary antibodies. Results indicated that serum from a patient with clinical signs of human ehrlichiosis reacted strongly to rP28 (31 kDa).

Example 2 Assaying for the presence of anti-OMP-1 antibody in a Patient

Convalescent-phase serum from a patient with clinical signs of human ehrlichiosis was reacted with the rP30 protein of E.canis as described in Example 1. The serum reacted strongly to rP30. These results indicate the rP30 is useful for diagnosing an infection with E. chafeensis in human patients.

Example 3. Identifying E. chafeensis-infected cells using anti-rP 28 antibody

E. chafeensis-infected DH82 cells were sonicated and centrifuged at 400 x g for 10 min. The supernatant was then centrifuged at 10,000 x g for 10 min to obtain ehrlichia-enriched pellet. The pellet was resuspended and incubated with rabbit anti-rP28 antibody or normal rabbit serum (1:100 dilution) at 37°C for 1h in PBS containing 1% bovine serum albumin (BSA-PBS). After washing, the ehrlichiae was incubated with gold-conjugated protein G (20 nm), Sigma) at 1:30 dilution for 1 h at room temperature in BSA-PBS. After washing again, the specimen was fixed with 1.25% formaldehyde, 2.5% glutaraldehyde, and 0.03% trinitrophenol in 0.1 M cacodylate buffer (pH 7.4) for 24h and postfixed in 1% osmium-1.5% potassium ferricyanide for 1 h (34). The section was then embedded in

PolyBed 812 (Polysciences, Warraington, Pa). The specimen was ultrathin sectioned at 60 nm, stained with uranyl acetate and lead citrate, and observed with a Philips 300 transmission electron microscope at 60 kV.

Transmission immunoelectron microscopy with colloidal gold-conjugated protein G and rabbit anti-rP28 antibody revealed gold particles bound to *E. chafeensis* surface. The distribution of the particles was random, close to the surface, and appeared as if almost embedded in the membrane, suggesting that the antigenic epitope protrudes very little from the lipid bilayer. Nonetheless, the antigenic epitope was surface-exposed, and thus, could be recognized by rabbit anti-rP28 antibody. No gold particles were observed on host cytoplasmic membrane or *E. chafeensis* incubated with normal rabbit serum.

Example 4. Immunization of mice and *E. chafeensis challenge*.

The rP28 band in SDS- PAGE was excised, minced, and mixed with an equal volume of Freund's incomplete or complete adjuvant. Nine BALB/c male mice (6 weeks old) were divided into two groups. Five mice were intraperitoneally immunized a total of four times at 10-day intervals; twice with a mixture of the minced gel with the r*P28* (30 to 40 µg of protein per mouse each time) and incomplete adjuvant, and twice with a mixture of the recombinant protein (the same amount as before) and complete adjuvant. Four mice were intraperitoneally injected with a mixture of the minced gel without protein and the respective adjuvants. For ehrlichia-challenge, approximately 1 x 10^7 DH82 cells heavily-infected with *E. chafeensis* were disrupted by sonication in serum-free DMEM (GIBCO-BRL) and centrifuged at 200 x g for 5 min. The supernatant was diluted to a final volume of 5 ml, and 0.3 ml was inoculated intraperitoneally into each mouse 10 days after the last immunization. Before challenge, all 5-immunized mice had a titer of 1:160 against *E. chafeensis* antigen by IFA and all 4-nonimmunized mice were negative.

At day 5 post-challenge, approximately 1 ml of blood was collected in an EDTA tube from each mouse and protection was assessed by PCR detection of *E. chafeensis* 16S rDNA in the buffy coat of the collected blood. *E. chafeensis* could not be reisolated in cell culture at day 10 postinfection . Day 5 post challenge is the optimum time at which establishment of ehrlichial infection can be examined by PCR without the influence of residual DNA from the ehrlichiae used as the challenge before the spontaneous clearance of organisms take place. The *E. chafeensis*-specific DNA fragment was observed in all nonimmunized mice but not in any immunized mice, indicating that immunization of rP28 apparently protects mice from ehrlichial infection and indicating that the P28 is a potential protective antigen

Example 5 Assaying for the presence of anti-P30 antibody in Dogs

The rP30 protein was used as an antigen in a Western immunoblot analysis and dot blot analysis to detect the presence of antibody to E. canis in serum from E-canis infected dogs. The results of the Western immunoblot analysis indicated that reactivity of the sera with rP30 was stronger than the reactivity that was observed when purified E.canis was used as antigen. The results of the dot blot assay indicated that rP30 is a useful and sensitive tool for serodiagnosis of canine ehrlichiosis.

CLAIMS

What is claimed is:

1. An isolated polynucleotide encoding an outer membrane protein of E. chafeensis or an immunogenic fragment thereof, wherein the outer membrane protein is selected from the group consisting of OMP-1, OMP-1B, OMP-1C, OMP-1D, OMP-1E, OMP-1F, OMP-1R, OMP-1S, OMP-1T, OMP-1U OMP-1V, OMP-1W OMP-1X OMP-1Y, and OMP-1Z.

2. The isolated polynucleotide of claim 1 wherein the polynucleotide encodes an OMP-1 protein comprising a sequence which is at least 85% homologous to the amino acid sequence shown in Fig. 3B, SEQ. ID NO ____.

3. The isolated polynucleotide of claim 1 wherein the polynucleotide encodes an OMP-1B protein comprising a sequence which is at least 85% homologous to the amino acid sequence shown in Fig. 4B, SEQ. ID NO ____.

4. The isolated polynucleotide of claim 1 wherein the polynucleotide encodes an OMP-1C protein comprising a sequence which is at least 85% homologous to the amino acid sequence shown in Fig.5B, SEQ. ID NO ____.

5. The isolated polynucleotide of claim 1 wherein the polynucleotide encodes an OMP-1D protein comprising a sequence which is at least 85% homologous to the amino acid sequence shown in Fig. 6B , SEQ. ID NO ____.

6. The isolated polynucleotide of claim 1 wherein the polynucleotide encodes an OMP-1E protein comprising a sequence which is at least 85% homologous to the amino acid sequence shown in Fig. 7B, SEQ. ID NO ____.

7. The isolated polynucleotide of claim 1 wherein the polynucleotide encodes an OMP-1F protein comprising a sequence which is at least 85% homologous to the amino acid sequence shown in Fig. 8B, SEQ. ID NO ____.

8. The isolated polynucleotide of claim 1 wherein the polynucleotide encodes an immunogenic fragment of the OMP-1 protein, said fragment comprising a sequence which is at least 85% homologous to the amino acid sequence extending from amino acid 6 through amino acid 251 as shown in Fig. 1, SEQ. ID NO ____.

9. An isolated polynucleotide encoding an outer membrane protein of E. canis or an immunogenic fragment thereof, wherein the outer membrane protein is selected from the group consisting of P30, P30-A, P30-1, P30-2, P30-3, P30-4, P30-5, P30-6, P30-7, P30-8, P30-9, P30-10.

10. The isolated polynucleotide of claim 9 wherein said P30 protein comprises a sequence which is at least 85% homologous to the amino acid sequence shown in Fig, 19 SEQ ID NO.

11. An isolated outer membrane protein of E. chafeensis or an immunogenic fragment thereof, wherein said protein is selected from the group consisting of OMP-1, OMP-1B, OMP-1C, OMP-1D, OMP-1E, OMP-1F, OMP-1R, OMP-1S, OMP-1T, OMP-1U OMP-1V, OMP-1W OMP-1X OMP-1Y, and OMP-1Z.

12. The isolated OMP-1 protein of claim 11, wherein said protein comprises a sequence which is at least 85% homologous to the amino acid sequence shown in Fig. 4B, SEQ. ID NO ____.

13. The isolated OMP-1B protein of claim 11 wherein said protein comprises a sequence which is at least 85% homologous to the amino acid sequence shown in Fig. 5B, SEQ. ID NO ____.

14. The isolated OMP-1C protein of claim 11 wherein said protein comprises a sequence which is at least 85% homologous to the amino acid sequence shown in Fig. 6B, SEQ. ID NO ____.

15. The isolated OMP-1D protein of claim 11 wherein said protein comprises a sequence which is at least 85% homologous to the amino acid sequence shown in Fig. 7B, SEQ. ID NO ____.

16. The isolated OMP-1E protein of claim 11 wherein said protein comprises a sequence which is at least 85% homologous to the amino acid sequence shown in Fig. 8B, SEQ. ID NO ____.

17. The isolated OMP-1F protein of claim 11 wherein said protein comprises a sequence which is at least 85% homologous to the amino acid sequence shown in Fig. 9B, SEQ. ID NO ____.

18. The isolated immunogenic fragment of the OMP-1 protein of claim 11, said fragment comprising a sequence which is at least 85% homologous to the amino acid sequence extending from amino acid 6 through amino acid 251 as shown in Fig. 1, SEQ. ID NO ____.

19. An isolated outer membrane protein of E. canis or an immunogenic fragment thereof, wherein the outer membrane protein is selected from the group consisting of P30, P30-A, P30-1, P30-2, P30-3, P30-4, P30-5, P30-6, P30-7, P30-8, P30-9, P30-10.

20. The isolated P-30 protein of claim 19 wherein said protein comprises a sequence which is at least 85% homologous to the amino acid sequence shown in Fig 19, SEQ ID NO. ___.

21. A method for diagnosing an infection with E. chafeensis in a patient comprising the steps of:

(a) providing a serum sample from the patient;

(b) providing an outer membrane protein selected from the group consisting of a protein of claim 11, a protein of claim 19, and mixtures thereof;

(c) contacting the serum sample with the outer membrane protein; and

(d) assaying for the formation of a complex between antibodies in the serum sample and the outer membrane protein, wherein formation of said complex is indicative of infection with E. chafeensis.

22. A method for diagnosing an infection with E. canis in a Canidae patient comprising the steps of:

(a) providing a serum sample from the patient ;

(b) providing an outer membrane protein of claim 19;

(c) contacting the serum sample with the outer membrane protein; and

(d) assaying for the formation of a complex between antibodies in the serum sample and the outer membrane protein, wherein formation of said complex is indicative of infection with E. canis.

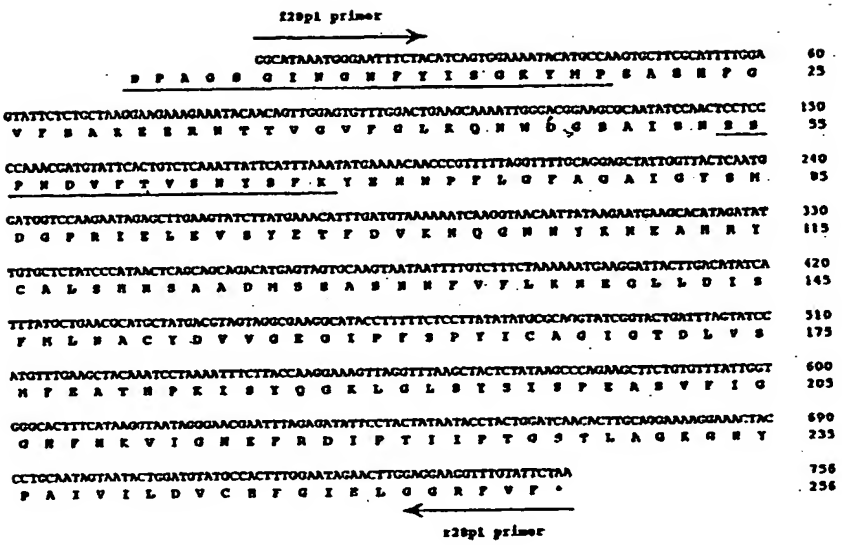

Fig. 1

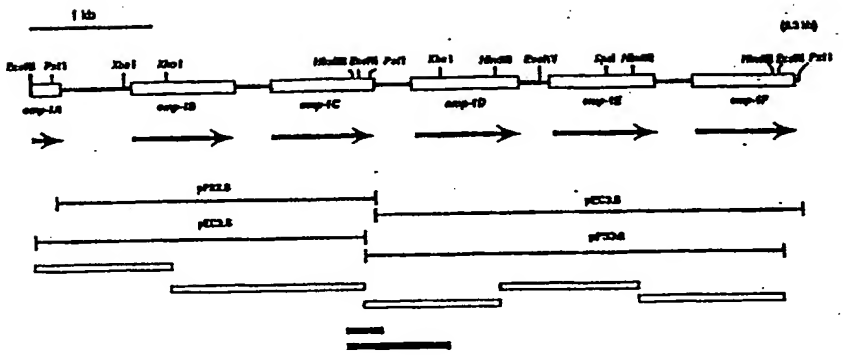

Fig. 2

```
        10         20         30         40         50         60
ATGAATTACA AAAAAGTTTT CATAACAAGT GCATTGATAT CATTAATATC TTCTCTACCT
        70         80         90        100        110        120
GGAGTATCAT TTTCCGACCC AGCAGGTAGT GGTATTAACG GTAATTTCTA CATCAGTGGA
       130        140        150        160        170        180
AAATACATGC CAAGTGCTTC GCATTTTGGA GTATTCTCTG CTAAGGAAGA AAGAAATACA
       190        200        210        220        230        240
ACAGTTGGAG TGTTTGGACT GAAGCAAAAT TGGGACGGAA GCGCAATATC CAACTCCTCC
       250        260        270        280        290        300
CCAAACGATG TATTCACTGT CTCAAATTAT TCATTTAAAT ATGAAAACAA CCCGTTTTTA
       310        320        330        340        350        360
GGTTTTGCAG GAGCTATTGG TTACTCAATG GATGGTCCAA GAATAGAGCT TGAAGTATCT
       370        380        390        400        410        420
TATGAAACAT TTGATGTAAA AAATCAAGGT AACAATTATA AGAATGAAGC ACATAGATAT
       430        440        450        460        470        480
TGTGCTCTAT CCCATAACTC AGCAGCAGAC ATGAGTAGTG CAAGTAATAA TTTTGTCTTT
       490        500        510        520        530        540
CTAAAAAATG AAGGATTACT TGACATATCA TTTATGCTGA ACGCATGCTA TGACGTAGTA
       550        560        570        580        590        600
GGCGAAGGCA TACCTTTTTC TCCTTATATA TGCGCAGGTA TCGGTACTGA TTTAGTATCC
       610        620        630        640        650        660
ATGTTTGAAG CTACAAATCC TAAAATTTCT TACCAAGGAA AGTTAGGTTT AAGCTACTCT
       670        680        690        700        710        720
ATAAGCCCAG AAGCTTCTGT GTTTATTGGT GGGCACTTTC ATAAGGTAAT AGGGAACGAA
       730        740        750        760        770        780
TTTAGAGATA TTCCTACTAT AATACCTACT GGATCAACAC TTGCAGGAAA AGGAAACTAC
       790        800        810        820        830        840
CCTGCAATAG TAATACTGGA TGTATGCCAC TTTGGAATAG AACTTGGAGG AAGGTTTGTA
       850        860        870        880        890        900
TTCTAA.... .......... .......... .......... .......... ..........
```

Fig. 3A

```
        10         20         30         40         50         60
MNYKKVFITS ALISLISSLP GVSFSDPAGS GINGNFYISG KYMPSASHFG VFSAKEERNT
        70         80         90        100        110        120
TVGVFGLKQN WDGSAISNSS PNDVFTVSNY SFKYENNPFL GFAGAIGYSM DGPRIELEVS
       130        140        150        160        170        180
YETFDVKNQG NNYKNEAHRY CALSHNSAAD MSSASNNFVF LKNEGLLDIS FMLNACYDVV
       190        200        210        220        230        240
GEGIPFSPYI CAGIGTDLVS MFEATNPKIS YQGKLGLSYS ISPEASVFIG GHFHKVIGNE
       250        260        270        280        290        300
FRDIPTIIPT GSTLAGKGNY PAIVILDVCH FGIEIGGRFV F..... ..........
```

Fig. 3B

```
        10         20         30         40         50         60
ATGAATTACA AGAAAATTTT TGTAAGCAGT GCATTAATTT CATTAATGTC AATCTTACCT
        70         80         90        100        110        120
TACCAATCTT TTGCAGATCC TGTAACTTCA AATGATACAG GAATCAACGA CAGCAGAGAA
       130        140        150        160        170        180
GGCTTCTACA TTAGTGTAAA GTATAATCCA AGCATATCAC ACTTCAGAAA ATTCTCAGCT
       190        200        210        220        230        240
GAAGAAGCTC CCATCAATGG AAATACTTCT ATCACTAAAA AGGTTTTCGG GCTGAAAAAA
       250        260        270        280        290        300
GACGGAGATA TAGCACAATC TGCGAATTTT AACAGGACAG ATCCAGCCCT CGAGTTTCAG
       310        320        330        340        350        360
AATAACCTAA TATCAGGATT CTCAGGAAGT ATTGGTTATG CTATGGATGG GCCAAGAATA
       370        380        390        400        410        420
GAACTTGAAG CTGCATACCA AAAATTTGAT GCAAAAAATC CTGACAACAA TGACACTAAT
       430        440        450        460        470        480
AGCGGTGACT ACTATAAATA CTTTGGACTA TCTCGTGAAG ACGCAATAGC AGATAAGAAA
       490        500        510        520        530        540
TATGTTGTCC TTAAAAATGA AGGCATCACT TTTATGTCAT TAATGGTTAA CACTTGCTAT
       550        560        570        580        590        600
GACATTACAG CTGAAGGAGT ACCTTTCATA CCGTATGCAT GTGCAGGTGT AGGAGCAGAC
       610        620        630        640        650        660
CTTATAAACG TATTTAAGGA TTTTAATTTA AAATTCTCAT ACCAAGGGAA AATAGGTATT
       670        680        690        700        710        720
AGCTATCCAA TCACACCAGA AGTTTCCGCT TTTATTGGAG GATACTACCA CGGAGTTATA
       730        740        750        760        770        780
GGAAATAATT TTAACAAAAT ACCTGTAATA ACACCTGTAG TATTAGAAGG AGCTCCTCAA
       790        800        810        820        830        840
ACCACATCTG CGCTAGTAAC TATTGACACT GGATACTTTG GCGGAGAAGT TGGAGTAAGG
       850        860        870        880        890        900
TTCACCTTCT AG..... ..... ..... ..... .....
```

Fig. 4A

```
        10         20         30         40         50         60
MNYKKIFVSS ALISLMSILP YQSFADPVTS NDTGINDSRE GFYISVKYNP SISHFRKFSA
        70         80         90        100        110        120
EEAPINGNTS ITKKVFGLKK DGDIAQSANF NRTDPALEFQ NNLISGFSGS IGYAMDGPRI
       130        140        150        160        170        180
ELEAAYQKFD AKNPDNNDTN SGDYYKYFGL SREDAIADKK YVVLKNEGIT FMSLMVNTCY
       190        200        210        220        230        240
DITAEGVPFI PYACAGVGAD LINVFKDFNL KFSYQGKIGI SYPITPEVSA FIGGYYEGVI
       250        260        270        280        290        300
GNNFNKIPVI TPVVLEGAPQ TTSALVTIDT GYFGGEVGVR FTF..... .....
```

Fig. 4B

```
        10          20          30          40          50          60
ATGAACTGCA AAAAATTTTT TATAACAACT GCATTGGCAT TGCCAATGTC TTTCTTACCT
        70          80          90         100         110         120
GGAATATTAC TTTCTGAACC AGTACAAGAT GACAGTGTGA GTGGCAATTT CTATATTAGT
       130         140         150         160         170         180
GGCAAGTACA TGCCAAGTGC TTCTCATTTT GGAGTTTTCT CTGCCAAAGA AGAAAAAAAT
       190         200         210         220         230         240
CCTACTGTCG CGTTGTATGG TTTGAAACAA GATTGGAACG GTGTTAGTGC TTCAAGTCAT
       250         260         270         280         290         300
GCTGATGCGG ACTTTAATAA CAAAGGTTAT TCTTTTAAAT ACGAAAACAA TCCATTTCTA
       310         320         330         340         350         360
GGTTTTGCAG GAGCTATTGG TTATTCAATG GGTGGTCCAA GAATAGAGTT TGAAGTGTCC
       370         380         390         400         410         420
TATGAAACAT TTGACGTGAA AAATCAAGGT GGTAATTACA AAAATGATGC TCACAGATAC
       430         440         450         460         470         480
TGTGCCTTAG ATCGTAAAGC AAGCAGCACT AATGCCACAG CTAGTCACTA CGTGCTACTA
       490         500         510         520         530         540
AAAAATGAAG GACTACTTGA TATATCACTT ATGTTGAATG CATGCTATGA CGTAGTAAGT
       550         560         570         580         590         600
GAAGGAATAC CTTTCTCTCC TTACATATGT GCAGGTGTTG GTACCGATTT AATATCCATG
       610         620         630         640         650         660
TTTGAAGCTA TAAACCCTAA AATTTCTTAT CAAGGAAAGT TAGGTTTGAG TTACTCTATA
       670         680         690         700         710         720
AACCCAGAAG CTTCTGTCTT TGTTGGTGGA CATTTTCATA AAGTTGCAGG TAATGAATTC
       730         740         750         760         770         780
AGGGACATTT CTACTCTTAA AGCGTTTGCT ACACCATCAT CTGCAGCTAC TCCAGACTTA
       790         800         810         820         830         840
GCAACAGTAA CACTGAGTGT GTGTCACTTT GGAGTAGAAC TTGGAGGAAG ATTTAACTTC
       850         860         870         880         890         900
TAA..... .......... ......  -- .......... .......... ..........
```

Fig. 5A

```
        10          20          30          40          50          60
MNCKKFFITT ALALPMSFLP GILLSEPVQD DSVSGNFYIS GKYMPSASHF GVFSAKEEKN
        70          80          90         100         110         120
PTVALYGLKQ DWNGVSASSH ADADFNNKGY SFKYENNPFL GFAGAIGYSM GGPRIEFEVS
       130         140         150         160         170         180
YETFDVKNQG GNYKNDAHRY CALDRKASST NATASHYVLL KNEGLLDISL MLNACYDVVS
       190         200         210         220         230         240
EGIPFSFYIC AGVGTDLISM FEAINPKISY QGKLGLSYSI NPEASVFVGG HFHKVAGNEF
       250         260         270         280         290         300
RDISTLKAFA TPSSAATPDL ATVTLSVCHF GVELGGRFNF .......... ..........
```

Fig. 5B

```
        10         20         30         40         50         60
ATGAACTGCG AAAAATTTTT TATAACAACT GCATTAACAT TACTAATGTC CTTCTTACCT
        70         80         90        100        110        120
GGAATATCAC TTTCTGATCC AGTACAGGAT GACAACATTA GTGGTAATTT CTACATCAGT
       130        140        150        160        170        180
GGAAAGTATA TGCCAAGCGC TTCGCATTTT GGAGTTTTTT CTGCCAAGGA AGAAAGAAAT
       190        200        210        220        230        240
ACAACAGTTG GAGTATTTGG AATAGAGCAA GATTGGGATA GATGTGTAAT ATCTAGAACC
       250        260        270        280        290        300
ACTTTAAGCG ATATATTCAC CGTTCCAAAT TATTCATTTA AGTATGAAAA TAATCTATTT
       310        320        330        340        350        360
TCAGGATTTG CAGGAGCTAT TGGCTACTCA ATGGATGGCC CAAGAATAGA GCTTGAAGTA
       370        380        390        400        410        420
TCTTATGAAG CATTCGATGT TAAAAATCAA GGTAACAATT ATAAGAACGA AGCACATAGA
       430        440        450        460        470        480
TATTATGCTC TGTCCCATCT TCTCGGCACA GAGACACAGA TAGATGGTGC AGGCAGTGCG
       490        500        510        520        530        540
TCTGTCTTTC TAATAAATGA AGGACTACTT GATAAATCAT TTATGCTGAA CGCATGTTAT
       550        560        570        580        590        600
GATGTAATAA GTGAAGGCAT ACCTTTTTCT CCTTATATAT GTGCAGGTAT TGGTATTGAT
       610        620        630        640        650        660
TTAGTATCCA TGTTTGAAGC TATAAATCCT AAAATTTCTT ATCAAGGAAA ATTAGGCTTA
       670        680        690        700        710        720
AGTTACCCTA TAAGCCCAGA AGCTTCTGTG TTTATTGGTG GACATTTTCA TAAGGTGATA
       730        740        750        760        770        780
GGAAACGAAT TTAGAGATAT TCCTACTATG ATACCTAGTG AATCAGCGCT TGCAGGAAAA
       790        800        810        820        830        840
GGAAACTACC CTGCAATAGT AACACTGGAC GTGTTCTACT TTGGCATAGA ACTTGGAGGA
       850        860        870        880        890        900
AGGTTTAACT TCCAACTTTG A......... .......... .......... ..........
```

Fig. 6A

```
        10         20         30         40         50         60
MNCEKFFITT ALTLLMSFLP GISLSDPVQD DNISGNFYIS GKYMPSASHF GVFSAKEERN
        70         80         90        100        110        120
TTVGVFGIEQ DWDRCVISRT TLSDIFTVPN YSFKYENNLF SGFAGAIGYS MDGPRIELEV
       130        140        150        160        170        180
SYEAFDVKNQ GNNYKNEAHR YYALSHLLGT ETQIDGAGSA SVFLINEGLL DKSFMLNACY
       190        200        210        220        230        240
DVISEGIPFS PYICAGIGID LVSMFEAINP KISYQGKLGL SYPISPEASV FIGGHFHKVI
       250        260        270        280        290        300
GNEFRDIPTM IPSESALAGK GNYPAIVTLD VFYFGIELGG RFNFQL.... ..........
```

Fig. 6B

```
        10         20         30         40         50         60
ATGAATTGCA AAAAATTTTT TATAACAACT GCATTAGTAT CACTAATGTC CTTTCTACCT
        70         80         90        100        110        120
GGAATATCAT TTTCTGATCC AGTGCAAGGT GACAATATTA GTGGTAATTT CTATGTTAGT
       130        140        150        160        170        180
GGCAAGTATA TGCCAAGTGC TTCGCATTTT GGCATGTTTT CTGCCAAAGA AGAAAAAAAT
       190        200        210        220        230        240
CCTACTGTTG CATTGTATGG CTTAAAACAA GATTGGGAAG GGATTAGCTC ATCAAGTCAC
       250        260        270        280        290        300
AATGATAATC ATTTCAATAA CAAGGGTTAT TCATTTAAAT ATGAAAATAA CCCATTTTTA
       310        320        330        340        350        360
GGGTTTGCAG GAGCTATTGG TTATTCAATG GGTGGTCCAA GAGTAGAGTT TGAAGTGTCC
       370        380        390        400        410        420
TATGAAACAT TTGACGTTAA AAATCAGGGT AATAACTATA AAAATGATGC TCACAGATAC
       430        440        450        460        470        480
TGTGCTTTAG GTCAACAAGA CAACAGCGGA ATACCTAAAA CTAGTAAATA CGTACTGTTA
       490        500        510        520        530        540
AAAAGCGAAG GATTGCTTGA CATATCATTT ATGCTAAATG CATGCTATGA TATAATAAAC
       550        560        570        580        590        600
GAGAGCATAC CTTTGTCTCC TTACATATGT GCAGGTGTTG GTACTGATTT AATATCCATG
       610        620        630        640        650        660
TTTGAAGCTA CAAATCCTAA AATTTCTTAC CAAGGGAAGT TAGGTCTAAG TTACTCTATA
       670        680        690        700        710        720
AACCCAGAAG CTTCTGTATT TATTGGTGGA CATTTTCATA AGGTGATAGG AAACGAATTT
       730        740        750        760        770        780
AGGGACATTC CTACTCTGAA AGCATTTGTT ACGTCATCAG CTACTCCAGA TCTAGCAATA
       790        800        810        820        830        840
GTAACACTAA GTGTATGTCA TTTTGGAATA GAACTTGGAG GAAGGTTTAA CTTCTAA...
```

Fig. 7A

```
        10         20         30         40         50         60
MNCKKFFITT ALVSLMSFLP GISFSDPVQG DNISGNFYVS GKYMPSASHF GMFSAKEEKN
        70         80         90        100        110        120
PTVALYGLKQ DWEGISSSSH NDNHFNNKGY SFKYENNPFL GFAGAIGYSM GGPRVEFEVS
       130        140        150        160        170        180
YETFDVKNQG NNYKNDAHRY CALGQQDNSG IPKTSKYVLL KSEGLLDISF MLNACYDIIN
       190        200        210        220        230        240
ESIPLSPYIC AGVGTDLISM FEATNPKISY QGKLGLSYSI NPEASVFIGG HFHKVIGNEF
       250        260        270        280        290        300
RDIPTLKAFV TSSATPDLAI VTLSVCHFGI ELGGRFNF.. .......... ..........
```

Fig. 7B

```
        10          20          30          40          50          60
ATGAATTGCA AAAAATTTTT TATAACAACT ACATTAGTAT CGCTAATGTC CTTCTTACCT
        70          80          90         100         110         120
GGAATATCAT TTTCTGATGC AGTACAGAAC GACAATGTTG GTGGTAATTT CTATATCAGT
       130         140         150         160         170         180
GGGAAATATG TACCAAGTGT TTCACATTTT GGCGTATTCT CTGCTAAACA GGAAAGAAAT
       190         200         210         220         230         240
ACAACAACCG GAGTATTTGG ATTAAAGCAA GATTGGGATG GCAGCACAAT ATCTAAAAAT
       250         260         270         280         290         300
TCTCCAGAAA ATACATTTAA CGTTCCAAAT TATTCATTTA AATATGAAAA TAATCCATTT
       310         320         330         340         350         360
CTAGGTTTTG CAGGAGCTGT TGGTTATTTA ATGAATGGTC CAAGAATAGA GTTAGAAATG
       370         380         390         400         410         420
TCCTATGAAA CATTTGATGT GAAAAACCAG GGTAATAACT ATAAGAACGA TGCTCACAAA
       430         440         450         460         470         480
TATTATGCTT TAACCCATAA CAGTGGGGGA AAGCTAAGCA ATGCAGGTGA TAAGTTTGTT
       490         500         510         520         530         540
TTTCTAAAAA ATGAAGGACT ACTTGATATA TCACTTATGT TGAATGCATG CTATGATGTA
       550         560         570         580         590         600
ATAAGTGAAG GAATACCTTT CTCTCCTTAC ATATGTGCAG GTGTTGGTAC TGATTTAATA
       610         620         630         640         650         660
TCCATGTTTG AAGCTATAAA CCCTAAAATT TCTTATCAAG GAAAGTTAGG TTTGAGTTAC
       670         680         690         700         710         720
TCCATAAGCC CAGAAGCTTC TGTTTTTGTT GGTGGACATT TTCATAAGGT GATAGGGAAT
       730         740         750         760         770         780
GAATTCAGAG ATATTCCTGC TATGATACCC AGTACCTCAA CTCTCACAGG TAATCACTTT
       790         800         810         820         830         840
ACTATAGTAA CACTAAGTGT ATGCCACTTT GGAGTGGAAC TTGGAGGAAG GTTTAACTTT
       850         860         870         880         890         900
TAA..... ..........  .......... .......... .......... ..........
```

Fig. 8A

```
        10          20          30          40          50          60
MNCKKFFITT TLVSLMSFLP GISFSDAVQN DNVGGNFYIS GKYVPSVSHF GVFSAKQERN
        70          80          90         100         110         120
TTTGVFGLKQ DWDGSTISKN SPENTFNVPN YSFKYENNPF LGFAGAVGYL MNGPRIELEM
       130         140         150         160         170         180
SYETFDVKNQ GNNYKNDAHK YYALTHNSGG KLSNAGDKFV FLKNEGLLDI SLMLNACYDV
       190         200         210         220         230         240
ISEGIPFSPY ICAGVGTDLI SMFEAINPKI SYQGKLGLSY SISPEASVFV GGHFHKVIGN
       250         260         270         280         290         300
EFRDIPAMIP STSTLTGNHF TIVTLSVCHF GVELGGRFNF .......... ..........
```

Fig. 8B

```
        10          20          30          40          50          60
ATGGAAAATC TCATGAATAA GAAAAACAAA TTCTTTACAA TAAGTACAGC AATGGTATGC
        70          80          90         100         110         120
TTATTGTTAT TACCTGGTAT ATCATTTTCA GAAACTATAA ACAACAGTGC TAAAAAACAG
       130         140         150         160         170         180
CCTGGGTTAT ATATCAGTGG GCAGTACAAA CCTAGTGTTT CAGTTTTTAG TAATTTTTCA
       190         200         210         220         230         240
GTAAAAGAAA CTAATGTTCC CACAAAGCAG TTAATAGCAC TTAAAAAAGA CATTAATTCT
       250         260         270         280         290         300
GTTGCAGTTG GTAGTAATGC TACTACAGGT ATTAGCAATC CAGGTAATTT CACAATTCCT
       310         320         330         340         350         360
TATACTGCAG AATTTCAAGA TAATGTTGCC AATTTCAATG GGGCTGTTGG TTACTCTTTT
       370         380         390         400         410         420
CCTGATAGTC TAAGAATTGA AATAGAGGGA TTTCATGAAA AATTTGATGT CAAAAACCCT
       430         440         450         460         470         480
GGAGGTTACA CACAAGTAAA AGATGCGTAC CGTTATTTTG CACTAGCACG TGATTTAAAA
       490         500         510         520         530         540
GATGGCTTCT TTGAACCTAA AGCGGAAGAT ACAGGTGTTT ATCATACTGT TATGAAAAAT
       550         560         570         580         590         600
GATGGATTAT CTATTTTATC TACTATGGTT AACGTCTGTT ACGATTTTTC TGTAGATGAA
       610         620         630         640         650         660
TTACCAGTCT TACCTTATAT ATGTGCAGGT ATGGGTATAA ACGCCATAGA ATTCTTCGAC
       670         680         690         700         710         720
GCTTTACATG TAAAATTTGC TTACCAAGGC AAACTAGGTA TTAGCTATCA ACTATTTACT
       730         740         750         760         770         780
AAAGTAAATT TATTCCTTGA TGGGTATTAC CATCAAGTAA TAGGCAATCA ATTCAAAAAC
       790         800         810         820         830         840
TTAAACGTAA ACCATGTTTA CACACTTAAA GAATCTCCTA AAGTCACATC TGCAGTAGCT
       850         860         870         880         890         900
ACACTTGACA TTGCATACTT TGGTGGCGAA GTTGGAATAA GATTCACATT TTAA......
```

Fig. 9A

```
        10          20          30          40          50          60
MVCLLLLPGI SFSETINNSA KKQPGLYISG QYKPSVSVFS NFSVKETNVP TKQLIALKKD
        70          80          90         100         110         120
INSVAVGSNA TTGISNPGNF TIPYTAEFQD NVANFNGAVG YSFPDSLRIE IEGFHEKFDV
       130         140         150         160         170         180
KNPGGYTQVK DAYRYFALAR DLKDGFFEPK AEDTGVYHTV MKNDGLSILS TMVNVCYDFS
       190         200         210         220         230         240
VDELPVLPYI CAGMGINAIE FFDALHVKFA YQGKLGISYQ LFTKVNLFLD GYYHQVIGNQ
       250         260         270         280         290         300
FKNLNVNHVY TLKESPKVTS AVATLDIAYF GGEVGIRFTF .......... ..........
```

Fig. 9B

```
        10         20         30         40         50         60
ATGATATATA AAGAAAAACT TACTAGAGTG GGAGAATATA TCTTAGCATA TTTATCATTT
        70         80         90        100        110        120
ATTCTTTCTA CTTATATCTT TCTAGTGCTG GTAAATATTA TTAGATATAA CAGCCTTGCT
       130        140        150        160        170        180
ATATGTGTTA TCAGTCTACT AAGAACTAAT ATCTTTAACG TTAGCACAAA AAAATTAATA
       190        200        210        220        230        240
AAAGATAAAT GTCGTGATAC TAAGTTTAGT AACATGAATT GTTATTTGTA CGGTAAACCG
       250        260        270        280        290        300
TTAAATTTAC AAATTTTTTA TGGAATATTT TCCTTTATTA GAAACTTTCA AAATAACACA
       310        320        330        340        350        360
CTAATAATTC CTAATGATAG TAAATGCGGC TTCTATACCA CGTTATGGGA TAATCCAGCA
       370        380        390        400        410        420
CTACATTATA CATATACACT TACTGGCAGT GAGTACCGTA ATTTTTTTGA CATTCTATAT
       430        440        450        460        470        480
GAAAACATTA TCTGTCAATG TAAATTACTT ATTAACTATA ACCGTTCTGT ATTAAACCAA
       490        500        510        520        530        540
CATAATAAAA ATACTCTCGT AATAATACCA ATACCTAATG CTAGAGAGTT CAGTAATGAA
       550        560        570        580        590        600
ATTCGAGTAA GGAATATATC AATAAATAAG GAAAGTTCTT ATGAGTGCTA A.........
```

Fig. 10A

```
        10         20         30         40         50         60
MIYKEKLTRV GEYILAYLSF ILSTYIFLVL VNIIRYNSLA ICVISLLRTN IFNVSTKKLI
        70         80         90        100        110        120
KDKCRDTKFS NMNCYLYGKP LNLQIFYGIF SFIRNFQNNT LIIPNDSKCG FYTTLWDNPA
       130        140        150        160        170        180
LHYTYTLTGS EYRNFFDILY ENIICQCKLL INYNRSVLNQ HNKNTLVIIP IPNAREFSNE
       190        200        210        220        230        240
IRVRNISINK ESSYEC.... .......... .......... .......... ..........
```

Fig. 10B

```
        10         20         30         40         50         60
ATGAATAAAA AAAACAAGTT TATTATAGCT ACAGCATTGG TATATTTACT GTCATTACCT
        70         80         90        100        110        120
AGTGTATCGT TTTCAGAGGT TACAAACAGC AGTATTAAAA AACACTCTGG GTTATATATT
       130        140        150        160        170        180
AGTGGACAAT ACAAACCAAG TGTTTCTGTT TTTAGTAGTT TCTCAATTAA AGAAACTAAC
       190        200        210        220        230        240
ACTATCACAA AAAATCTTAT AGCGTTAAAA AAAGATATTA ACTCTCTTGA AGTTAACGCC
       250        260        270        280        290        300
GATGCTAGTC AAGGTATTAG TCATCCAGGA AATTTTACTA TACCTTATAT AGCAGCATTT
       310        320        330        340        350        360
GAAGATAATG CTTTTAATTT CAACGGTGCT ATTGGTTACA TTACTGAAGG TCTAAGGATT
       370        380        390        400        410        420
GAAATAGAAG GTTCCTATGA AGAATTTGAT GCTGAAAACC CTGGAGGTTA TGGTCTAAAT
       430        440        450        460        470        480
GATGCCTTTC GGTACTTTGC TTTAGCACGT GATATGGAAA GCAACAAGTT CCTACCAAAA
       490        500        510        520        530        540
GCACAAAGCT CAC..... .......... .......... .......... ..........
```

Fig. 11A

```
        10         20         30         40         50         60
MNKKNKFIIA TALVYLLSLP SVSFSEVTNS SIKKHSGLYI SGQYKPSVSV FSSFSIKETN
        70         80         90        100        110        120
TITKNLIALK KDINSLEVNA DASQGISHPG NFTIPYIAAF EDNAFNFNGA IGYITEGLRI
       130        140        150        160        170        180
EIEGSYEEFD AENPGGYGLN DAFRYFALAR DMESNKFLPK AQSS..... ..........
```

Fig. 11B

```
        10         20         30         40         50         60
TCTAGAATAC ATGATGAAAA TTATGCTATT ACAACAAATA ATAAATTATC CATCGCATCT
        70         80         90        100        110        120
ATTATGGTTA ACACCTGCTA TGATATTTCA ATTAATAATA CATCAATAGT ACCGTATTTA
       130        140        150        160        170        180
TGCACAGGCA TTGGTGAAGA TCTTGTAGGG CTTTTTAATA CAATACATTT TAAACTTGCA
       190        200        210        220        230        240
TATCAAGGGA AAGTTGGAAT GAGTTATTTG ATAAATAACA ATATCCTATT ATTTTCTGAC
       250        260        270        280        290        300
ATATATTATC ATAAAGTCAT GGGTAACAGA TTTAAAAATT TGTACATGCA ATATGTAGCT
       310        320        330        340        350        360
GATCCTAATA TTTCTGAAGA AACTATACCT ATATTAGCAA AACTTGATAT TGGTTATTTT
       370        380        390        400        410        420
GGAAGTGAAA TTGGAATAAG GTTTATGTTT AACTAA.... .......... ..........
```

Fig. 12A

```
        10         20         30         40         50         60
SRIHDENYAI TTNNKLSIAS IMVNTCYDIS INNTSIVPYL CTGIGEDLVG LFNTIHFKLA
        70         80         90        100        110        120
YQGKVGMSYL INNNILLFSD IYYHKVMGNR FKNLYMQYVA DPNISEETIP ILAKLDIGYF
       130        140        150        160        170        180
GSEIGIRFMF N.......... .......... .......... .......... ..........
```

Fig. 12B

```
        10          20          30          40          50          60
ATGACAAAGA AATTTAATTT TGTAAATGTT ATATTAACAT TTTTGTTATT TCTTTTCCCA
        70          80          90         100         110         120
CTTAAGTCAT TTACAACATA TGCAAATAAT AACACAATCA CTCAAAAAGT TGGATTGTAC
       130         140         150         160         170         180
ATAAGTGGTC AATATAAGCC AAGTATTCCT CATTTCAAGA ATTTTTCAGT AGAAGAAAAT
       190         200         210         220         230         240
GACAAAGTAG TAGATTTGAT AGGTCTTACA ACTGATGTTA CATATATCAC AGAACATATA
       250         260         270         280         290         300
TTACGAGATA ATACAAAATT CAACACTCAT TATATTGCAA AGTTCAAGAA CAATTTTATA
       310         320         330         340         350         360
AATTTCAGCA GTGCAATTGG TTATTATTCT GGGCAAGGAC CAAGGTTAGA AATAGAAAGC
       370         380         390         400         410         420
TCTTATGGGG ATTTTGATGT TGTAAATTAT AAAAATTATG CAGTACAAGA TGTTAATAGA
       430         440         450         460         470         480
TATTTTGCTT TAGTACGTGA AAAAAATGGT TCAAATTTCT CTCCAAAACC ACATGAAACT
       490         500         510         520         530         540
AGTCAACCCT CTGACAGTAA TCCTAAAAAG TCTTTTTATA CTTTAATGAA GAATAATGGG
       550         560         570         580         590         600
GTATTTGTTG CATCAGTAAT AATCAACGGT TGTTATGATT TTTCTTTTAA TAACACAACA
       610         620         630         640         650         660
ATATCACCTT ACGTATGTAT AGGAGTTGGA GGAGATTTTA TAGAGTTTTT TGAAGTAATG
       670         680         690         700         710         720
CATATCAAGT TTGCTTGCCA AAGTAAGGTT GGTATTAGCT ATCCAATATC TCCCTCTATT
       730         740         750         760         770         780
ACTATTTTTG CTGATGCACA TTATCACAAG GTCATAAATA ATAAATTTAA CAACCTACAT
       790         800         810         820         830         840
GTTAAGTATT CATATGAACT TAAAAACTCA CCTACCATTA CCTCTGCAAC AGCCAAACTA
       850         860         870         880         890         900
AACATTGAAT ATTTTGGTGG TGAAGTTGGG ATGAGATTTA TATTTTAA.. ..........
```

Fig. 13A

```
        10          20          30          40          50          60
MTKKFNFVNV ILTFLLFLFP LKSFTTYANN NTITQKVGLY ISGQYKPSIP HFKNFSVEEN
        70          80          90         100         110         120
DKVVDLIGLT TDVTYITEHI LRDNTKFNTH YIAKFKNNFI NFSSAIGYYS GQGPRLEIES
       130         140         150         160         170         180
SYGDFDVVNY KNYAVQDVNR YFALVREKNG SNFSPKPHET SQPSDSNPKK SFYTLMKNNG
       190         200         210         220         230         240
VFVASVIING CYDFSFNNTT ISPxVCIGVG GDFIEFFEVM HIKFACQSKV GISYPISPSI
       250         260         270         280         290         300
TIFADAHYHK VINNKFNNLH VKYSYELKNS PTITSATAKL NIEYFGGEVG MRFIF.....
```

Fig. 13B

```
      10         20         30         40         50         60
ATGAGCAAAA AAAAGTTTAT TACAATAGGA ACAGTACTTG CATCTCTATT ATCATTCTTA
      70         80         90        100        110        120
TCTATTGAAT CCTTTTCAGC TATAAATCAT AATCATACAG GAAATAACAC TAGTGGTATA
     130        140        150        160        170        180
TATATTACAG GGCAGTATAG ACCAGGAGTA TCCCATTTTA GCAATTTCTC AGTAAAAGAA
     190        200        210        220        230        240
ACTAATGTTG ATACAATACA ACTAGTAGGA TATAAAAAAA GTGCGTCTTC TATCGATCCT
     250        260        270        280        290        300
AACACTTATT CAAACTTTCA AGGTCCATAT ACTGTTACAT TTCAAGATAA TGCTGCTAGT
     310        320        330        340        350        360
TTCAGTGGAG CAATTGGATA TTCTTACCCC GAAAGTCTAA GACTTGAACT TGAAGGTTCT
     370        380        390        400        410        420
TACGAAAAAT TTGATGTCAA AGATCCTAAA GACTACTCAG CAAAAGATGC TTTTAGGTTT
     430        440        450        460        470        480
TTTGCTCTAG CACGTAATAC GTCTACTACT GTTCCTGATG CTCAAAAATA TACAGTTATG
     490        500        510        520        530        540
AAGAATAATG GCTTATCTGT TGCATCAATC ATGATCAATG GTTGTTATGA TCTATCTTTT
     550        560        570        580        590        600
AATAATTTAG TCGTATCACC TTATATATGT GCAGGTATTG GTGAAGATTT CATTGAATTT
     610        620        630        640        650        660
TTTGATACTT TGCACATTAA ACTTGCTTAT CAAGGAAAAC TAGGTATTAG TTATTACTTC
     670        680        690        700        710        720
TTTCCTAAGA TTAATGTATT TGCTGGTGGG TACTATCATA GAGTTATAGG GAATAAATTT
     730        740        750        760        770        780
AAAAATTTAA ATGTTAACCA TGTTGTTACA CTTGATGAAT TTCCTAAAGC AACTTCTGCA
     790        800        810        820        830        840
GTAGCTACAC TTAATGTTGC TTATTTTGGT GGTGAAGCTG GAGTAAAGTT TACATTTTAA
     850        860        870        880        890        900
.......... .......... .......... .......... .......... ..........
```

Fig. 14A

```
      10         20         30         40         50         60
MSKKKFITIG TVLASLLSFL SIESFSAINH NHTGNNTSGI YITGQYRPGV SHFSNFSVKE
      70         80         90        100        110        120
TNVDTIQLVG YKKSASSIDP NTYSNFQGPY TVTFQDNAAS FSGAIGYSYP ESLRLELEGS
     130        140        150        160        170        180
YEKFDVKDPK DYSAKDAFRF FALARNTSTT VPDAQKYTVH KNNGLSVASI MINGCYDLSF
     190        200        210        220        230        240
NNLVVSPYIC AGIGEDFIEF FDTLHIKLAY QGKLGISYYF FPKINVFAGG YYHRVIGNKF
     250        260        270        280        290        300
KNLNVNHVVT LDEFPKATSA VATLNVAYFG GEAGVKFTF. .......... ..........
```

Fig. 14B

```
        10          20          30          40          50          60
ATGAGTGCTA AAAAAAAGCT TTTTATAATA GGGTCAGTGT TAGTATGTTT AGTGTCATAC
        70          80          90         100         110         120
TTACCTACTA AATCTTTGTC AAACTTAAAT AATATTAATA ATAACACTAA GTGCACTGGG
       130         140         150         160         170         180
CTATATGTCA GTGGACAATA TAAACCTACT GTTTCTCACT TTAGTAATTT TTCACTTAAA
       190         200         210         220         230         240
GAAACTTATA CTGACACTAA AGAGTTATTA GGACTAGCAA AAGATATTAA GTCTATTACA
       250         260         270         280         290         300
GATATAACAA CAAATAAAAA ATTCAACATT CCTTATAACA CAAAATTTCA AGATAATGCT
       310         320         330         340         350         360
GTTAGCTTCA GTGCAGCTGT TGGATATATT TCCCAAGACA GTCCAAGGGT TGAGGTAGAA
       370         380         390         400         410         420
TGGTCTTATG AAGAATTTGA CGTTAAAAAT CCTGGTAATT ACGTAGTAAG TGAAGCCTTC
       430         440         450         460         470         480
AGGTATATTG CTTTAGCAAG AGGAATTGAT AATCTTCAAA AATATCCTGA AACAAATAAG
       490         500         510         520         530         540
TATGTTGTTA TAAAGAACAA TGGCTTATCT GTCGCATCCA TTATAATCAA TGGCTGTTAT
       550         560         570         580         590         600
GATTTTTCTT TAAACAATTT AAAAGTATCA CCTTACATAT GCGTAGGGTT TGGTGGGGAC
       610         620         630         640         650         660
ATTATAGAAT TTTTTAGTGC TGTAAGTTTT AAATTTGCTT ATCAAGGTAA GGTAGGTATC
       670         680         690         700         710         720
AGTTATCCAT TATTCTCTAA TATGATTATA TTTGCTGACG GATATTACCA TAAGGTCATA
       730         740         750         760         770         780
GGAAATAAAT TTAACAATTT AAATGTTCAA CACGTTGTTA GTCTTAACAG TCATCCTAAG
       790         800         810         820         830         840
TCTACTTTTG CAGTAGCTAC TCTTAATGTT GAGTATTTCG GTAGTGAATT TGGGTTAAAA
       850         860         870         880         890         900
TTTATATTTT AA..... ..... ..... ..... .....
```

Fig. 15A

```
        10          20          30          40          50          60
MSAKKKLFII GSVLVCLVSY LPTKSLSNLN NINNNTKCTG LYVSGQYKPT VSHFSNFSLK
        70          80          90         100         110         120
ETYTDTKELL GLAKDIKSIT DITTNKKFNI PYNTKFQDNA VSFSAAVGYI SQDSPRVEVE
       130         140         150         160         170         180
WSYEEFDVKN PGNYVVSEAF RYIALARGID NLQKYPETNK YVVIKNNGLS VASIIINGCY
       190         200         210         220         230         240
DFSLNNLKVS PYICVGFGGD IIEFFSAVSP KFAYQGKVGI SYPLFSNMII FADGYYHKVI
       250         260         270         280         290         300
GNKFNNLNVQ HVVSLNSHPK STFAVATLNV EYFGSEFGLK FIF..... .....
```

Fig. 15B

```
        10         20         30         40         50         60
ATGAGTAAAA AAAATTTTAT TACAATAGGA GCAACACTTA TTCATATGTT GTTACCTAAC
        70         80         90        100        110        120
ATATCTTTTC CAGAAACTAT TAACAATAAC ACTGATAAAC TTTCTGGGTT ATATATAAGT
       130        140        150        160        170        180
GGGCAATATA AACCAGGGAT TTCTCATTTC AGCAAATTTT CAGTCAAAGA AATCTATAAT
       190        200        210        220        230        240
GATAACATTC AACTAATTGG GTTAAGACAC AACGCAATTT CTACTAGTAC CCTTAATATT
       250        260        270        280        290        300
AATACAGATT TTAATATCCC CTATAAAGTA ACATTTCAAA ATAACATTAC CAGCTTTAGT
       310        320        330        340        350        360
GGAGCTATTG GTTATTCTGA TCCCACAGGG GCAAGATTTG AGCTTGAAGG TTCTTATGAA
       370        380        390        400        410        420
GAATTTGATG TGACAGATCC TGGAGACTGC TTAATAAAAG ATACCTATAG ATATTTCGCT
       430        440        450        460        470        480
TTAGCTAGAA ACCCATCAGG TTCTAGCCCT ACCTCAAACA ACTATACTGT TATGAGAAAT
       490        500        510        520        530        540
GATGGTGTTT CCATTACTTC TGTTATATTT AATGGCTGTT ATGACATCTT TTTAAAGGAT
       550        560        570        580        590        600
TTAGAAGTAT CACCTTATGT ATGTGTTGGT GTAGGTGGAG ATTTTATAGA ATTTTTTGAC
       610        620        630        640        650        660
GCATTACACA TTAAATTAGC ATACCAAGGC AAGTTAGGTA TCAATTATCA CTTATCGACT
       670        680        690        700        710        720
CAAGCAAGCG TATTTATTGA TGGATATTAT CATAAGGTTA TAGGAAATCA ATTCAACAAT
       730        740        750        760        770        780
CTAAATGTTC AACACGTGGC TAGTACAGAT TTTGGACCTG TATACGCAGT AGCCACACTT
       790        800        810        820        830        840
AACATTGGTT ATTTTGGTGG TGAAATCGGA ATTAGACTTA CATTTTAA.. ..........
```

Fig. 16A

```
        10         20         30         40         50         60
MSKKNFITIG ATLIHMLLPN ISFPETINNN TDKLSGLYIS GQYKPGISHF SKFSVKEIYN
        70         80         90        100        110        120
DNIQLIGLRH NAISTSTLNI NTDFNIPYKV TFQNNITSFS GAIGYSDPTG ARFELEGSYE
       130        140        150        160        170        180
EFDVTDPGDC LIKDTYRYFA LARNPSGSSP TSNNYTVMRN DGVSITSVIF NGCYDIFLKD
       190        200        210        220        230        240
LEVSPYVCVG VGGDFIEFFD ALHIKLAYQG KLGINYHLST QASVFIDGYY HKVIGNQFNN
       250        260        270        280        290        300
LNVQHVASTD FGPVYAVATL NIGYFGGEIG IRLTF..... ..........
```

Fig. 16B

```
      10         20         30         40         50         60
ATGAATAATA GAAAAAGTTT TTTTATAATA GGTGCATCAT TACTAGCAAG CTTATTATTC
      70         80         90        100        110        120
ACATCTGAGG CCTCTTCTAC AGGAAATGTA AGTAACCATA CTTATTTTAA ACCTAGGTTA
     130        140        150        160        170        180
TATATCAGTG GACAATATAG ACCAGGAGTT TCTCATTTTA GCAAATTTTC AGTCAAAGAA
     190        200        210        220        230        240
ACCAACTACA ATACTACTCA ACTAGTTGGG CTTAAAAAGG ACATCAGTGT CATAGGGAAC
     250        260        270        280        290        300
AGTAATATCA CAACCTACAC AAATTTCAAC TTTCCTTACA TTGCAGAATT TCAAGACAAT
     310        320        330        340        350        360
GCCATAAGTT TCAGTGGGGC AATTGGATAC TTGTATTCCG AGAATTTTAG AATTGAAGTA
     370        380        390        400        410        420
GAGGCTTCTT ATGAAGAATT TGATGTTAAA AATCCAGAAG GATCTGCTAC AGACGCATAC
     430        440        450        460        470        480
AGGTATTTTG CACTAGCACG TGCTATGGAT GGCACTAATA AATCTAGTCC TGATGACACA
     490        500        510        520        530        540
AGAAAATTCA CTGTCATGAG AAATGACGGG TTATCAATTT CATCAGTAAT GATAAATGGG
     550        560        570        580        590        600
TGTTACAATT TTACATTAGA TGATATACCA GTAGTACCGT ATGTATGCGC AGGAATAGGA
     610        620        630        640        650        660
GGAGATTTCA TAGAGTTTTT TAATGATTTA CATGTTAAGT TTGCTCATCA AGGCAAGGTA
     670        680        690        700        710        720
GGTATTAGTT ATTCTATATC CCCTGAAGTA AGTTTATTTC TTAACGGATA TTACCATAAA
     730        740        750        760        770        780
GTAACAGGTA ACAGATTTAA AAACTTACAC GTTCAACACG TAAGTGATTT AAGTGACGCT
     790        800        810        820        830        840
CCTAAGTTCA CATCTGCAGT TGCTACACTC AATGTTGGGT ACTTTGGTGG CGAAATTGGA
     850        860        870        880        890        900
GTAAGATTTA TATTTTAA.. .......... .......... .......... ..........
```

Fig. 17A

```
      10         20         30         40         50         60
MNNRKSFFII GASLLASLLF TSEASSTGNV SNHTYFKPRL YISGQYRPGV SHFSKFSVKE
      70         80         90        100        110        120
TNYNTTQLVG LKKDISVIGN SNITTYTNFN FPYIAEFQDN AISFSGAIGY LYSENFRIEV
     130        140        150        160        170        180
EASYEEFDVK NPEGSATDAY RYFALARAMD GTNKSSPDDT RKFTVMRNDG LSISSVMING
     190        200        210        220        230        240
CYNFTLDDIP VVPYVCAGIG GDFIEFFNDL HVKFAHQGKV GISYSISPEV SLFLNGYYHK
     250        260        270        280        290        300
VTGNRFKNLH VQHVSDLSDA PKFTSAVATL NVGYFGGEIG VRFIF..... ..........
```

Fig. 17B

```
      10         20         30         40         50         60
TAGCAGCACT AAAAAACAGT TTGGGTTATA TGTTAGTGGA CAACACCAGC CTAGTGTTTC
      70         80         90        100        110        120
TATTTTTAGC AATTTCTCAG TAAAGGAAAC TAATTTTCCT ACAAAGTATT CTAGCAGCTT
     130        140        150        160        170        180
CTTAAAAAAA GACATTAATT CTGTCGAATT TGACGATAGT GTTACTGCTG GCATTAGTTA
     190        200        210        220        230        240
CCCACTTAAT TTCAGTACTC CTTATATAGC TGTATTTCAA GATAATATTT CTAATTTTAA
     250        260        270        280        290        300
TGGCGCTATT GGGTACACTT TTGTTGAAGG CCCAAGAATT GAAATAGAAG GTTCTTATGA
     310        320        330        340        350        360
AGAATTCGAT GTCAAAGACC CTGGAAGATA TACAGAAATA CAAGATGCAT ACCGTTACTT
     370        380        390        400        410        420
TGCTTTAGCA CGTGATATAG ACTCTATTCC TACTAGCCCA AAAAATAGAA CTTCACATGA
     430        440        450        460        470        480
TGGCAACAGT TCATATAAGG TATACCACAC TGTAATGAAA AATGAAGGAC TATCTATAAT
     490        500        510        520        530        540
ATCCATTATG GTCAATGGCT GCTATGATTT TTCTTCAGAT AATTTATCAA TATTACCTTA
     550        560        570        580        590        600
TGTATGTGGT GGTATAGGTG TAAATGCTAT AGAGTTTTTC GATGCATTAC ATGTTAAATT
     610        620        630        640        650        660
CGCGTGTCAG GGTAAATTAG GTATTACTTA TCCATTATCT TCCAACGTTA GTTTATTTGC
     670        680        690        700        710        720
TGGTGGATAT TATCACCAAG TAATGGGCAA CCAATTTAAA AATCTAAATG TTCAACATGT
     730        740        750        760        770        780
AGCTGAACTT AATGACGCAC CCAAAGTTAC ATCTGCAGTA GCTACACTTG ACATTGGGTA
     790        800        810        820        830        840
TTTTGGTGGT GAAATTGGAG CAAGGCTTAT ATTTTAA... .......... ..........
```

Fig. 18A

```
      10         20         30         40         50         60
SSTKKQFGLY VSGQHQPSVS IFSNFSVKET NFPTKYSSSF LKKDINSVEF DDSVTAGISY
      70         80         90        100        110        120
PLNFSTPYIA VFQDNISNFN GAIGYTFVEG PRIEIEGSYE EFDVKDPGRY TEIQDAYRYF
     130        140        150        160        170        180
ALARDIDSIP TSPKNRTSHD GNSSYKVYHT VMKNEGLSII SIMVNGCYDF SSDNLSILPY
     190        200        210        220        230        240
VCGGIGVNAI EFFDALHVKF ACQGKLGITY PLSSNVSLFA GGYYHQVMGN QFKNLNVQHV
     250        260        270        280        290        300
AELNDAPKVT SAVATLDIGY FGGEIGARLI F..........  ..........
```

Fig. 18B

```
        10         20         30         40         50         60
ATGAATTGCA AAAGATTTTT CATAGCAAGT GCATTGATAT CACTAATGTC TTTCTTACCT
        70         80         90        100        110        120
AGCGTATCTT TTTCTGAATC AATACATGAA GATAATATAA ATGGTAACTT TTACATTAGT
       130        140        150        160        170        180
GCAAAGTATA TGCCAAGTGC CTCACACTTT GGCGTATTTT CAGTTAAAGA AGAGAAAAAC
       190        200        210        220        230        240
ACAACAACTG GAGTTTTCGG ATTAAAACAA GATTGGGACG GAGCAACAAT AAAGGATGCA
       250        260        270        280        290        300
AGCAGCAGCC ACACAATAGA CCCAAGTACA ATATTCTCCA TTTCAAATTA TTCATTTAAA
       310        320        330        340        350        360
TATGAAAACA ATCCATTTTT AGGGTTTGCA GGAGCTATTG GCTACTCAAT GGGTGGTCCA
       370        380        390        400        410        420
AGGGTAGAGT TTGAAGTGTC TTACGAAATA TTTGATGTAA AAAACCAAGG TAACAGTTAC
       430        440        450        460        470        480
AAGAACGATG CTCACAAATA TTGCGCTTTA TCAAGACACA CCGGAGGTAT GCCACAAGCC
       490        500        510        520        530        540
GGTCATCAAA ATAAATTTGT CTTCCTAAAA AATGAAGGAT TACTTGACAT ATCACTTATG
       550        560        570        580        590        600
ATAAACGCAT GTTATGATAT AACAATCGAC AGCATGCCAT TTTCTCCATA TATATGTGCA
       610        620        630        640        650        660
GGTATTGGTA GTGACTTAGT TTCGATGTTT GAAACTACAA ATCCTAAAAT TTCTTATCAA
       670        680        690        700        710        720
GGAAAATTAG GTGTAAGTTA CTCCATAAGC CCAGAAGCAT CTGTTTTTGT TGGAGGACAC
       730        740        750        760        770        780
TTTCACAGAG TTATAGGTAA TGAATTTAAA GACATTCCTG CAATAACTCC TGCTGGAGCA
       790        800        810        820        830        840
ACAGAAATTA AAGGCACACA GTTTACAACA GTAACATTAA ACATATGCCA CTTCGGACTA
       850        860        870        880        890        900
GAGCTTGGAG GCAGGTTTAC TTTTTAA... .......... .......... ..........
```

Fig. 19A

```
        10         20         30         40         50         60
MNCKRFFIAS ALISLMSFLP SVSFSESIHE DNINGNFYIS AKYMPSASHF GVFSVKEEKN
        70         80         90        100        110        120
TTTGVFGLKQ DWDGATIKDA SSSHTIDPST IFSISNYSFK YENNPFLGFA GAIGYSMGGP
       130        140        150        160        170        180
RVEFEVSYEI FDVKNQGNSY KNDAHKYCAL SRHTGGMPQA GHQNKFVFLK NEGLLDISLM
       190        200        210        220        230        240
INACYDITID SMPFSPYICA GIGSDLVSMF ETTNPKISYQ GKLGVSYSIS PEASVFVGGH
       250        260        270        280        290        300
FHRVIGNEFK DIPAITPAGA TEIKGTQFTT VTLNICHFGL ELGGRFTF.. ..........
```

Fig. 19B

```
        10         20         30         40         50         60
ATGAAATATA AAAAAACTTT TACAGTAACT GCATTAGTAT TATTAACTTC CTTTACACAT
        70         80         90        100        110        120
TTTATACCTT TTTATAGTCC AGCACGTGCC AGTACAATTC ACAACTTCTA CATTAGTGGA
       130        140        150        160        170        180
AAATATATGC CAACAGCGTC ACATTTTGGA ATTTTTTCAG CTAAAGAAGA ACAAAGTTTT
       190        200        210        220        230        240
ACTAAGGTAT TAGTTGGGTT AGATCAACGA TTATCACATA ATATTATAAA CAATAATGAT
       250        260        270        280        290        300
ACAGCAAAGA GTCTTAAGGT TCAAAATTAT TCATTTAAAT ACAAAAATAA CCCATTTCTA
       310        320        330        340        350        360
GGATTTGCAA GAGCTATTGG TTATTCAATA GGCAATTCAA GAATAGAACT AGAAGTATCA
       370        380        390        400        410        420
CATGAAATAT TTGATACTAA AAACCCAGGA AACAATTATT TAAATGACTC TCACAAATAT
       430        440        450        460        470        480
TGCGCTTTAT CTCATGGAAG TCACATATGC AGTGATGGAA ATAGCGGAGA TTGGTACACT
       490        500        510        520        530        540
GCAAAAACTG ATAAGTTTGT ACTTCTGAAA AATGAAGGTT TACTTGACGT CTCATTTATG
       550        560        570        580        590        600
TTAAACGCAT GTTATGACAT AACAACTGAA AAAATGCCTT TTTCACCTTA TATATGTGCA
       610        620        630        640        650        660
GGTATTGGTA CTGATCTCAT ATCTATGTTT GAGACAACAC AAAACAAAAT ATCTTATCAA
       670        680        690        700        710        720
GGAAAGTTAG GTTTAAACTA TACTATAAAC TCAAGAGTTT CTGTTTTTGC AGGTGGGCAC
       730        740        750        760        770        780
TTTCATAAAG TAATAGGTAA TGAATTTAAA GGTATTCCTA CTCTATTACC TGATGGATCA
       790        800        810        820        830        840
AACATTAAAG TACAACAGTC TGCAACAGTA ACATTAGATG TGTGCCATTT CGGGTTAGAG
       850        860        870        880        890        900
ATTGGAAGTA GATTTTTCTT TTAA...... .......... .......... ..........
```

Fig. 20A

```
        10         20         30         40         50         60
MKYKKTFTVT ALVLLTSFTH FIPFYSPARA STIHNFYISG KYMPTASHFG IFSAKEEQSF
        70         80         90        100        110        120
TKVLVGLDQR LSHNIINNND TAKSLKVQNY SFKYKNNPFL GFARAIGYSI GNSRIELEVS
       130        140        150        160        170        180
HEIFDTKNPG NNYLNDSHKY CALSHGSHIC SDGNSGDWYT AKTDKFVLLK NEGLLDVSFM
       190        200        210        220        230        240
LNACYDITTE KMPFSPYICA GIGTDLISMF ETTQNKISYQ GKLGLNYTIN SRVSVFAGGH
       250        260        270        280        290        300
FHKVIGNEFK GIPTLLPDGS NIKVQQSATV TLDVCHFGLE IGSRFFF... ..........
```

Fig. 20B

```
        10         20         30         40         50         60
ATGTTTTATA CTAATATATA TATTCTGGCT TGTATTTACT TTGCACTTCC ACTATTGTTA
        70         80         90        100        110        120
ATTTATTTTC ACTATTTTAG GTGTAATATG AATTGCAAAA AAATTCTTAT AACAACTGCA
       130        140        150        160        170        180
TTAATATCAT TAATGTACTC TATTCCAAGC ATATCTTTTT CTGATACTAT ACAAGATGGT
       190        200        210        220        230        240
AACATGGGTG GTAACTTCTA TATTAGTGGA AAGTATGTAC CAAGTGTCTC ACATTTTGGT
       250        260        270        280        290        300
AGCTTCTCAG CTAAAGAAGA AAGCAAATCA ACTGTTGGAG TTTTTGGATT AAAACATGAT
       310        320        330        340        350        360
TGGGATGGAA GTCCAATACT TAAGAATAAA CACGCTGACT TTACTGTTCC AAACTATTCG
       370        380        390        400        410        420
TTCAGATACG AGAACAATCC ATTTCTAGGG TTTGCAGGAG CTATCGGTTA CTCAATGGGT
       430        440        450        460        470        480
GGCCCAAGAA TAGAATTCGA AATATCTTAT GAAGCATTCG ACGTAAAAAG TCCTAATATC
       490        500        510        520        530        540
AATTATCAAA ATGACGCGCA CAGGTACTGC GCTCTATCTC ATCACACATC GGCAGCCATG
       550        560        570        580        590        600
GAAGCTGATA AATTTGTCTT CTTAAAAAAC GAAGGGTTAA TTGACATATC ACTTGCAATA
       610        620        630        640        650        660
AATGCATGTT ATGATATAAT AAATGACAAA GTACCTGTTT CTCCTTATAT ATGCGCAGGT
       670        680        690        700        710        720
ATTGGTACTG ATTTGATTTC TATGTTTGAA GCTACAAGTC CTAAAATTTC CTACCAAGGA
       730        740        750        760        770        780
AAACTGGGCA TTAGTTACTC TATTAATCCG GAAACCTCTG TTTTCATCGG TGGGCATTTC
       790        800        810        820        830        840
CACAGGATCA TAGGTAATGA GTTTAGAGAT ATTCCTGCAA TAGTACCTAG TAACTCAACT
       850        860        870        880        890        900
ACAATAAGTG GACCACAATT TGCAACAGTA ACACTAAATG TGTGTCACTT TGGTTTAGAA
       910        920        930        940        950        960
CTTGGAGGAA GATTTAACTT CTAA..... ..........  ..........  ..........
```

Fig. 21A

```
        10         20         30         40         50         60
MFYTNIYILA CIYFALPLLL FYFHYFRCNM NCKKILITTA LISLMYSIPS ISFSDTIQDG
        70         80         90        100        110        120
NMGGNFYISG KYVPSVSHFG SFSAKEESKS TVGVFGLKHD WDGSPILKNK HADFTVPNYS
       130        140        150        160        170        180
FRYENNPFLG FAGAIGYSMG GPRIEFEISY EAFDVKSPNI NYQNDAHRYC ALSHHTSAAM
       190        200        210        220        230        240
EADKFVFLKN EGLIDISLAI NACYDIINDK VPVSFYICAG IGTDLISMFE ATSPKISYQG
       250        260        270        280        290        300
KLGISYSINP ETSVFIGGHF HRIIGNEFRD IPAIVPSNST TISGPQFATV TLNVCHFGLE
       310        320        330        340        350        360
LGGRFNF... ..........  ..........  ..........  ..........  ..........
```

Fig. 21B

```
        10         20         30         40         50         60
ATGAATTGCA AAAAAATTCT TATAACAACT GCATTAATGT CATTAATGTA CTATGCTCCA
        70         80         90        100        110        120
AGCATATCTT TTTCTGATAC TATACAAGAC GATAACACTG GTAGCTTCTA CATCAGTGGA
       130        140        150        160        170        180
AAATATGTAC CAAGTGTTTC ACATTTTGGT GTTTTCTCAG CTAAAGAAGA AAGAAACTCA
       190        200        210        220        230        240
ACTGTTGGAG TTTTTGGATT AAAACATGAT TGGAATGGAG GTACAATATC TAACTCTTCT
       250        260        270        280        290        300
CCAGAAAATA TATTCACAGT TCAAAATTAT TCGTTTAAAT ACGAAAACAA CCCATTCTTA
       310        320        330        340        350        360
GGGTTTGCAG GAGCTATTGG TTATTCAATG GGTGGCCCAA GAATAGAACT TGAAGTTCTG
       370        380        390        400        410        420
TACGAGACAT TCGATGTGAA AAATCAGAAC AATAATTATA AGAACGGCGC ACACAGATAC
       430        440        450        460        470        480
TGTGCTTTAT CTCATCATAG TTCAGCAACA AACATGTCCT CCGCAAGTAA CAAATTTGTT
       490        500        510        520        530        540
TTCTTAAAAA ATGAAGGGTT AATTGACTTA TCATTTATGA TAAATGCATG CTATGACATA
       550        560        570        580        590        600
ATAATTGAAG GAATGCCTTT TTCACCTTAT ATTTGTGCAG GTGTTGGTAC TGATGTTGTT
       610        620        630        640        650        660
TCCATGTTTG AAGCTATAAA TCCTAAAATT TCTTACCAAG GAAAACTAGG ATTAGGTTAT
       670        680        690        700        710        720
AGTATAAGTT CAGAAGCCTC TGTTTTTATC GGTGGACACT TTCACAGAGT CATAGGTAAT
       730        740        750        760        770        780
GAATTTAGAG ACATCCCTGC TATGGTTCCT AGTGGATCAA ATCTTCCAGA AAACCAATTT
       790        800        810        820        830        840
GCAATAGTAA CACTAAATGT GTGTCACTTT GGTTTAGAAC TTGGAGGAAG ATTTAACTTC
       850        860        870        880        890        900
TGA..... .......... .......... .......... .......... ..........
```

Fig. 22A

```
        10         20         30         40         50         60
MNCKKILITT ALMSLMYYAP SISFSDTIQD DNTGSFYISG KYVPSVSHFG VFSAKEERNS
        70         80         90        100        110        120
TVGVFGLKHD WNGGTISNSS PENIFTVQNY SFKYENNPFL GFAGAIGYSM GGPRIELEVL
       130        140        150        160        170        180
YETFDVKNQN NNYKNGAHRY CALSHHSSAT NMSSASNKFV FLKNEGLIDL SFMINACYDI
       190        200        210        220        230        240
IIEGMPFSPY ICAGVGTDVV SMFEAINPKI SYQGKLGLGY SISSEASVFI GGHFHRVIGN
       250        260        270        280        290        300
EERDIPAMVP SGSNLPENQF AIVTLNVCHF GLELGGRFNF .......... ..........
```

Fig. 22B

```
        10          20          30          40          50          60
ATGAATTGTA AAAAAGTTTT CACAATAAGT GCATTGATAT CATCCATATA CTTCCTACCT
        70          80          90         100         110         120
AATGTCTCAT ACTCTAACCC AGTATATGGT AACAGTATGT ATGGTAATTT TTACATATCA
       130         140         150         160         170         180
GGAAAGTACA TGCCAAGTGT TCCTCATTTT GGAATTTTTT CAGCTGAAGA AGAGAAAAAA
       190         200         210         220         230         240
AAGACAACTG TAGTATATGG CTTAAAAGGA AAACTGGCAG GAGATGCAAT ATCTAGTCAA
       250         260         270         280         290         300
AGTCCAGATG ATAATTTTAC CATTCGAAAT TACTCATTCA AGTATGCAAG CAACAAGTTT
       310         320         330         340         350         360
TTAGGGTTTG CAGTAGCTAT TGGTTACTCG ATAGGCAGTC CAAGAATAGA AGTTGAGATG
       370         380         390         400         410         420
TCTTATGAAG CATTTGATGT GAAAAATCCA GGTGATAATT ACAAAAACGG TGCTTACAGG
       430         440         450         460         470         480
TATTGTGCTT TATCTCATCA AGATGATGCG GATGATGACA TGACTAGTGC AACTGACAAA
       490         500         510         520         530         540
TTTGTATATT TAATTAATGA AGGATTACTT AACATATCAT TTATGACAAA CATATGTTAT
       550         560         570         580         590         600
GAAACAGCAA GCAAAAATAT ACCTCTCTCT CCTTACATAT GTGCAGGTAT TGGTACTGAT
       610         620         630         640         650         660
TTAATTCACA TGTTTGAAAC TACACATCCT AAAATTTCTT ATCAAGGAAA GCTAGGGTTG
       670         680         690         700         710         720
GCCTACTTCG TAAGTGCAGA GTCTTCGGTT TCTTTTGGTA TATATTTTCA TAAAATTATA
       730         740         750         760         770         780
AATAATAAGT TTAAAAATGT TCCAGCCATG GTACCTATTA ACTCAGACGA GATAGTAGGA
       790         800         810         820         830         840
CCACAGTTTG CAACAGTAAC ATTAAATGTA TGCTACTTTG GATTAGAACT TGGATGTAGG
       850         860         870         880         890         900
TTCAACTTCT AA..... ..... ..... ..... .....
```

Fig. 23A

```
        10          20          30          40          50          60
MNCKKVFTIS ALISSIYFLP NVSYSNPVYG NSMYGNFYIS GKYMPSVPHF GIFSAEEEKK
        70          80          90         100         110         120
KTTVVYGLKG KLAGDAISSQ SPDDNFTIRN YSFKYASNKF LGFAVAIGYS IGSPRIEVEM
       130         140         150         160         170         180
SYEAFDVKNP GDNYKNGAYR YCALSHQDDA DDDMTSATDK FVYLINEGLL NISFMTNICY
       190         200         210         220         230         240
ETASKNIPLS PYICAGIGTD LIHMFETTHP KISYQGKLGL AYFVSAESSV SFGIYFHKII
       250         260         270         280         290         300
NNKFKNVPAM VPINSDEIVG PQFATVTLNV CYFGLELGCR FNF..... .....
```

Fig. 23B

```
        10         20         30         40         50         60
ATGAACTGTA AAAAATTTCT TATAACAACT ACATTGGTAT CACTAACAAT TCTTTTACCT
        70         80         90        100        110        120
GGCATATCTT TCTCCAAACC AATACATGAA AACAATACTA CAGGAAACTT TTACATTATT
       130        140        150        160        170        180
GGAAAATATG TACCAAGTAT TTCACATTTT GGGAACTTTT CAGCTAAAGA AGAAAAAAAC
       190        200        210        220        230        240
ACAACTACTG GAATTTTTGG ATTAAAAGAA TCATGGACTG GTGGTATCAT CCTTGATAAA
       250        260        270        280        290        300
GAACATGCAG CTTTTAATAT CCCAAATTAT TCATTTAAAT ATGAAAATAA TCCATTTTTA
       310        320        330        340        350        360
GGATTTGCAG GGGTAATTGG CTATTCAATA GGTAGTCCAA GAATAGAATT TGAAGTATCA
       370        380        390        400        410        420
TACGAGACAT TCGATGTACA AAATCCAGGA GATAAGTTTA ACAATGATGC ACATAAGTAT
       430        440        450        460        470        480
TGTGCTTTAT CCAATGATTC CAGTAAAACA ATGAAAAGTG GTAAATTCGT TTTTCTCAAA
       490        500        510        520        530        540
AATGAAGGAT TAAGTGACAT ATCACTCATG TTAAATGTAT GTTATGATAT AATAAACAAA
       550        560        570        580        590        600
AGAATGCCTT TTTCACCTTA CATATGTGCA GGCATTGGTA CTGACTTAAT ATTCATGTTT
       610        620        630        640        650        660
GACGCTATAA ACCATAAAGC TGCTTATCAA GGAAAATTAG GTTTTAATTA TCCAATAAGC
       670        680        690        700        710        720
CCAGAAGCTA ACATTTCTAT GGGTGTGCAC TTTCACAAAG TAACAAACAA CGAGTTTAGA
       730        740        750        760        770        780
GTTCCTGTTC TATTAACTGC TGGAGGACTC GCTCCAGATA ATCTATTTGC AATAGTAAAG
       790        800        810        820        830        840
TTGAGTATAT GTCATTTTGG GTTAGAATTT GGGTACAGGG TCAGTTTTTA A.........
```

Fig. 24A

```
        10         20         30         40         50         60
MNCKKFLITT TLVSLTILLP GISFSKPIHE NNTTGNFYII GKYVPSISHF GNFSAKEEKN
        70         80         90        100        110        120
TTTGIFGLKE SWTGGIILDK EHAAFNIPNY SFKYENNPFL GFAGVIGYSI GSPRIEFEVS
       130        140        150        160        170        180
YETFDVQNPG DKFNNDAHKY CALSNDSSKT MKSGKFVFLK NEGLSDISLM LNVCYDIINK
       190        200        210        220        230        240
RMPFSPYICA GIGTDLIFMF DAINHKAAYQ GKLGFNYPIS PEANISMGVH FHKVTNNEFR
       250        260        270        280        290        300
VPVLLTAGGL APDNLFAIVK LSICHFGLEF GYRVSF.... .......... ..........
```

Fig. 24B

```
      10         20         30         40         50         60
ATGAATAATA AACTCAAATT TACTATAATA AACACAGTAT TAGTATGCTT ATTGTCATTA
      70         80         90        100        110        120
CCTAATATAT CTTCCTCAAA GGCCATAAAC AATAACGCTA AAAAGTACTA CGGATTATAT
     130        140        150        160        170        180
ATCAGTGGAC AATATAAACC CAGTGTTTCT GTTTTCAGTA ATTTTTCAGT TAAAGAAACC
     190        200        210        220        230        240
AATGTCATAA CTAAAAACCT TATAGCTTTA AAAAAAGATG TTGACTCTAT TGAAACCAAG
     250        260        270        280        290        300
ACTGATGCCA GTGTAGGTAT TAGTAACCCA TCAAATTTTA CTATCCCCTA TACAGCTGTA
     310        320        330        340        350        360
TTTCAAGATA ATTCTGTCAA TTTCAATGGA ACTATTGGTT ACACCTTTGC TGAAGGTACA
     370        380        390        400        410        420
AGAGTTGAAA TAGAAGGTTC TTATGAGGAA TTTGATGTTA AAAACCCTGG AGGCTATACA
     430        440        450        460        470        480
CTAAGTGATG CCTATCGCTA TTTTGCATTA GCACGTGAAA TGAAAGGTAA TAGTTTTACA
     490        500        510        520        530        540
CCTAAAGAAA AAGTTTCTAA TAGTATTTTT CACACTGTAA TGAGAAATGA TGGATTATCT
     550        560        570        580        590        600
ATAATATCTG TTATAGTAAA TGTTTGCTAC GATTTCTCTT TGAACAATTT GTCAATATCG
     610        620        630        640        650        660
CCTTACATAT GTGGAGGAGC AGGGGTAGAT GCTATAGAAT TCTTCGATGT ATTACACATT
     670        680        690        700        710        720
AAGTTTGCAT ATCAAAGCAA GCTAGGTATT GCTTATTCTC TACCATCTAA CATTAGTCTC
     730        740        750        760        770        780
TTTGCTAGTT TATATTACCA TAAAGTAATG GGCAATCAAT TTAAAAATTT AAATGTCCAA
     790        800        810        820        830        840
CATGTTGCTG AACTTGCAAG TATACCTAAA ATTACATCCG CAGTTGCTAC ACTTAATATT
     850        860        870        880        890        900
GGTTATTTTG GAGGTGAAAT TGGTGCAAGA TTGACATTTT AA..... .....
```

Fig. 25A

```
      10         20         30         40         50         60
MNNKLKFTII NTVLVCLLSL PNISSSKAIN NNAKKYYGLY ISGQYKPSVS VFSNFSVKET
      70         80         90        100        110        120
NVITKNLIAL KKDVDSIETK TDASVGISNP SNFTIPYTAV FQDNSVNFNG TIGYTFAEGT
     130        140        150        160        170        180
RVEIEGSYEE FDVKNPGGYT LSDAYRYFAL AREMKGNSFT PKEKVSNSIF HTVMRNDGLS
     190        200        210        220        230        240
IISVIVNVCY DFSLNNLSIS PYICGGAGVD AIEFFDVLHI KFAYQSKLGI AYSLPSNISL
     250        260        270        280        290        300
FASLYYHKVM GNQFKNLNVQ HVAELASIPK ITSAVATLNI GYFGGEIGAR LTF.....
```

Fig. 25B

```
        10         20         30         40         50         60
ATGGCAAATT TTATGTACAA AAAATACAAA CTAATGACAG CAGGTGTAGT ATTATTTCAC
        70         80         90        100        110        120
ATGTTATTTC TACCTCATGT TTCTTTCGCA AAAAATACAA ACAGCAATAA ACTTGGATTA
       130        140        150        160        170        180
TACATCAGTG GACAGTATAA CCCTAGTGTT TCTGTTTTTA GCAATTTTTC AGCAAAAGAA
       190        200        210        220        230        240
ACCAATGTTC ATACAGTACA ACTCATGGCG CTTAAAAAAG ACATTGATTC TATTGAAGTT
       250        260        270        280        290        300
GATACTGGAA ATAGCGCAGG TATTAGCAAA CCACAAAATT TCACAGTTCT TTATACTCCA
       310        320        330        340        350        360
AAATTTCAAG ATAATGTTGC TGGTCTTAGC GGTGCACTTG GATTCTTTTA TTCTAAAGGA
       370        380        390        400        410        420
TTAAGGATTG AAATGGGGTT TTCTTATGAA AAATTTGATG CTAAAGACCT TGGTGAGTAC
       430        440        450        460        470        480
ACCAAAATAA AAGATGCTTA TAGATATTTT GCTCTAGTAC GTGAAATGCA TGTTAGTCTC
       490        500        510        520        530        540
ATTTATCCAA AAGATAATAA CACAGGAACA CATTATACTG TTATGAGAAA TGATGGTATA
       550        560        570        580        590        600
TCTATTTCTT CTGCTACAGT AAATGGCTGC TATGATTCTT TTTTCCAGTT TATCTTTGTC
       610        620        630        640        650        660
ACCTATATGT GTATAGGCAT CGGTATAGAT GCTATAGAAT TTCTTAATGC ATACATATTA
       670        680        690        700        710        720
AGTTTGCTTG CCAAGGTAGT TAAGGTGTTA ACTTATTCTG TATCTCCCAA TGTTAATTTA
       730        740        750        760        770        780
TTTGCAGATG GATATTATCA TAAAGTGATG GGCAATAAAT TTAAAAATTT ACCTGTTCAA
       790        800        810        820        830        840
TACGTTAATA CTTTAGAAGA GTATCCAAGA GTTACATCTG CAATTGCTAC ACTTGATATT
       850        860        870        880        890        900
GGCTACCTCG GTGGTGAAAT TGGCATAAGA TTTATATTTT AA..... .....
```

Fig. 26A

```
        10         20         30         40         50         60
MYKKYKLMTA GVVLFHMLFL PHVSFAKNTN SNKLGLYISG QYNPSVSVFS NFSAKETNVH
        70         80         90        100        110        120
TVQLMALKKD IDSIEVDTGN SAGISKPQNF TVLYTPKFQD NVAGLSGALG FFYSKGLRIE
       130        140        150        160        170        180
MGFSYEKFDA KDLGEYTKIK DAYRYFALVR EMHVSLIYPK DNNTGTHYTV MRNDGISISS
       190        200        210        220        230        240
ATVNGCYDSF FQFIFVTYMC IGIGIDAIEF LNAYILSLLA KVVKVLTYSV SPNVNLFADG
       250        260        270        280        290        300
YYHKVMGNKF KNLPVQYVNT LEEYPRVTSA IATLDIGYLG GEIGIRFIF. .....
```

Fig. 26B

```
        10          20          30          40          50          60
ATGGGAAATT CTATGAATAA TAAAAGTCAA TTCTTAATAA GATTTATATT TTTAACATGC
        70          80          90         100         110         120
ATGCTGTCAT TACCTAATAT ATCTCTTTCA AAAGTAAATA ACGAAAAACA TTCTGGTTTG
       130         140         150         160         170         180
TATATTAGCG GGCAATACAA ACCCAGTGTT TCTGTTTTCA GTAATTTTTC AGTTAAAGAA
       190         200         210         220         230         240
ACCAACTTTC ATACAAAACA TCTCATAGCT CTTAAACAAG ATGTTGATTC TGTTGAAATT
       250         260         270         280         290         300
GATACTGGTA GTAATACAGC AGGTATTAGT AACCCATCTA ACTTTACAAT CCCTTATACT
       310         320         330         340         350         360
GCAGAATTTC AAGACAACCA TACTAACTGC AATGGCTCTA TTGGTTATGC TTTTGCTGAA
       370         380         390         400         410         420
GGTCCAAGAA TTGAAATAGA ATTATCATAT GAAAAATTTG ATGTTAAAAA TCCCACAGGG
       430         440         450         460         470         480
TATACTACAG TAAAAGATGC TTATAGATAC TTTGCTTTAG CACGTGAAAT AAATATTTCT
       490         500         510         520         530         540
CTATTCCAAC CAAAACAAAA AGAAGGTAGT GGAATTTACC ATGTCGTAAT GAAAAACGAT
       550         560         570         580         590         600
GGGTTATCTA TCTTATCCAA TATAGTTAAT ATTTGCTACG ATTTTTCTTT AAATAATTTA
       610         620         630         640         650         660
CCTATATCAC CTTATTTATG CGGAGGAATG GGTATAAATG CCATAGAATT CTTTGACGCT
       670         680         690         700         710         720
TTACATGTGA AATTTGCTTA TCAAAGCAAG GCAGGAATTA GTTATCAACT ATTACGTAAA
       730         740         750         760         770         780
ATCAACTTAT TTATTGATGT ATATTACTAC GAAGTAATAA GTAATAAATT TAAAAACCTG
       790         800         810         820         830         840
AAAGTCCAAC ATGTACATGA ACTTAAAGAT AATCCAAAAG TCACATCTGC AGTTGCTACA
       850         860         870         880         890         900
CTTGATATAG CATATTTTGG TAGTGAAGCT GGCATAAGAA TTATATTTTA A.........
```

Fig. 27A

```
        10          20          30          40          50          60
MNNKSQFLIR FIFLTCMLSL PNISLSKVNN EKHSGLYISG QYKPSVSVFS NFSVKETNFH
        70          80          90         100         110         120
TKHLIALKQD VDSVEIDTGS NTAGISNPSN FTIPYTAEFQ DNHTNCNGSI GYAFAEGPRI
       130         140         150         160         170         180
EIELSYEKFD VKNPTGYTTV KDAYRYFALA REINISLFQP KQKEGSGIYH VVMKNDGLSI
       190         200         210         220         230         240
LSNIVNICYD FSLNNLPISP YLCGGMGINA IEFFDALHVK FAYQSKAGIS YQLLRKINLF
       250         260         270         280         290         300
IDVYYYEVIS NKFKNLKVQH VHELKDNPKV TSAVATLDIA YFGSEAGIRI IF.........
```

Fig. 27B

```
        10         20         30         40         50         60
ATGAATAGCA AGAGTAAGTT CTTTACAATA TGTACATCGT TAATATGCTT ATTATCATCA
        70         80         90        100        110        120
CCTAACACAT CTCTCTCAAA CTTCATAGGC AATAGTACAA AACATTCTGG ATTATATGTT
       130        140        150        160        170        180
AGCGGACAAT ATAAGCCCAG CGTTTCCATT TTTAGCAAAT TTTCAGTAAA AGAAACAAAT
       190        200        210        220        230        240
ACACATACAG TACAGTTAGT AGCTCTTAAA AAAGATGTTA ATTCTATTTC TATGAACATC
       250        260        270        280        290        300
AGTAATGGTG CTACAGGCAT TAGCAAAGCA ACAAATTTTA ATCTTCCTTA TGTTGCAGAA
       310        320        330        340        350        360
TTTCAAGACA ATGCCTTCAA CTTCAGTGGA GCTATTGGTT ATTCACTTTT TGAACAACTA
       370        380        390        400        410        420
AACATTGAAG TTGAAGGTTC TTATGAAGAA TTCGATGCCA AAAATCCTGG TGGTTATATT
       430        440        450        460        470        480
TTAAATGATG CATTCCGCTA TTTTGCATTG GCACGTGAAA TGGGACAAGA AAAAAATGAT
       490        500        510        520        530        540
AATAAGCATC TTAGTCCTAA GGAGGAGCAT GATATAAGTA AAACATATTA CACAGTCATG
       550        560        570        580        590        600
AGAAATAATG GGTTATCTAT ATTATCTATT ATGATAAATG GCTGCTATAA TCTACCTCTC
       610        620        630        640        650        660
AATGATTTAT CAATATCACC TTATTTTTGT ACAGGAATAG GTGTAGATGC TATAGAATTT
       670        680        690        700        710        720
TTTGATGCAC TGCATCTTAA ACTTGCTTTG CAAAGTAAAA TAGGAGCTAC TTACCAATTA
       730        740        750        760        770        780
TCAGACAACA TTAGTTTATT TACAAATGGA TATTACCATC AAGTAATAGG TGATCAATTT
       790        800        810        820        830        840
AAAAACTTAA AAGTCCAATA TATAGGTGAA CTTAAAGAGA ACCCGAAAAT TACATCTGCA
       850        860        870        880        890        900
GTTGCTACTC TCAATGTTGG ATACTTTGGA GGTGAAATTG GAGTAAGACT CACACTTTAA
       910        920        930        940        950        960
.......... .......... .......... .......... .......... ..........
```

Fig. 28A

```
        10         20         30         40         50         60
MNSKSKFFTI CTSLICLLSS PNTSLSNFIG NSTKHSGLYV SGQYKPSVSI FSKFSVKETN
        70         80         90        100        110        120
THTVQLVALK KDVNSISMNI SNGATGISKA TNFNLPYVAE FQDNAFNFSG AIGYSLFEQL
       130        140        150        160        170        180
NIEVEGSYEE FDAKNPGGYI LNDAFRYFAL AREMGQEKND NKHLSPKEEH DISKTYYTVM
       190        200        210        220        230        240
RNNGLSILSI MINGCYNLPL NDLSISPYFC TGIGVDAIEF FDALHLKLAL QSKIGATYQL
       250        260        270        280        290        300
SDNISLFTNG YYHQVIGDQF KNLKVQYIGE LKENPKITSA VATLNVGYFG GEIGVRLTL.
```

Fig. 28B

```
        10          20          30          40          50          60
AAGCTTCTTA TGAAGAATTT GACGTTAAAA ATCCTGAAGG ATCTACTACA GACTCCTATA
        70          80          90         100         110         120
GATATTTCGC GTTAGCACGT GGCATGGATG GTAATAATAT TCCTACAAGT CAAAAATTTA
       130         140         150         160         170         180
CTGTAATGAG AAACGACGGG TTATTAATCT CATCTGTTAT GATAAATGGC TGTTACAATG
       190         200         210         220         230         240
TCATACTAAA TGATATACAA GCAGAACCTT ACATATGTGC AGGACTAGGA GGAGATTTTA
       250         260         270         280         290         300
TAGAATTCTT CAATGGCTTT CATGTTAAGC TAGCTTATCA AGGTAAAGTA GGCATTAGTT
       310         320         330         340         350         360
ATCAAATATT CCCTGAAGTA AGATTATTTA TTGATGGATA CTACCATAAA GTAAAAGGCA
       370         380         390         400         410         420
ACAAGTTTAA AAATTTACAC GTTCAACATG TAGGTGCACT TGCAGCACTC CCTAAAGTTA
       430         440         450         460         470         480
CATCTGCAGT TGCAACACTT AATATTGGAT ACTTTGGTTG TGAAGCTGGA GTAAGATTCA
       490         500         510         520         530         540
TATTTTAA.. .......... .......... .......... .......... ..........
```

Fig. 29A

```
        10          20          30          40          50          60
ASYEEFDVKN PEGSTTDSYR YFALARGMDG NNIPTSQKFT VMRNDGLLIS SVMINGCYNV
        70          80          90         100         110         120
ILNDIQAEPY ICAGLGGDFI EFFNGFHVKL AYQGKVGISY QIFPEVRLFI DGYYHKVKGN
       130         140         150         160         170         180
KFKNLHVQHV GALAALPKVT SAVATLNIGY FGCEAGVRFI F.......... ..........
```

Fig. 29B

```
        10          20          30          40          50          60
ATGAATTATA AGAAAATTCT AGTAAGAAGC GCGTTAATCT CATTAATGTC AATCTTACCA
        70          80          90         100         110         120
TATCAGTCTT TTGCAGATCC TGTAGGTTCA AGAACTAATG ATAACAAAGA AGGCTTCTAC
       130         140         150         160         170         180
ATTAGTGCAA AGTACAATCC AAGTATATCA CACTTTAGAA AATTCTCTGC TGAAGAAACT
       190         200         210         220         230         240
CCTATTAATG GAACAAATTC TCTCACTAAA AAAGTTTTCG GACTAAAGAA AGATGGTGAT
       250         260         270         280         290         300
ATAACAAAAA AAGACGATTT TACAAGAGTA GCTCCAGGCA TTGATTTTCA AAATAACTTA
       310         320         330         340         350         360
ATATCAGGAT TTTCAGGAAG TATTGGTTAC TCTATGGACG GACCAAGAAT AGAACTTGAA
       370         380         390         400         410         420
GCTGCATATC ACAATTTAAT CCAAAAACAC GATAACAATG ATACTGATAA TGGTGAATAC
       430         440         450         460         470         480
TATAAACATT TTGCATATCT CGTAAAGATG CCATGGAAGA TCAGCCATAT GTTGTTCTTA
       490         500         510         520         530         540
AAAATGACGG CATAC..... .......... .......... .......... ..........
```

Fig. 30A

```
        10          20          30          40          50          60
MNYKKILVRS ALISLMSILP YQSFADPVGS RTNDNKEGFY ISAKYNPSIS HFRKFSAEET
        70          80          90         100         110         120
PINGTNSLTK KVFGLKKDGD ITKKDDFTRV APGIDFQNNL ISGFSGSIGY SMDGPRIELE
       130         140         150         160         170         180
AAYHNLIQKH DNNDTDNGEY YKHFAYLVKM PWKISHMLFL KMTAY..... ..........
```

Fig. 30B

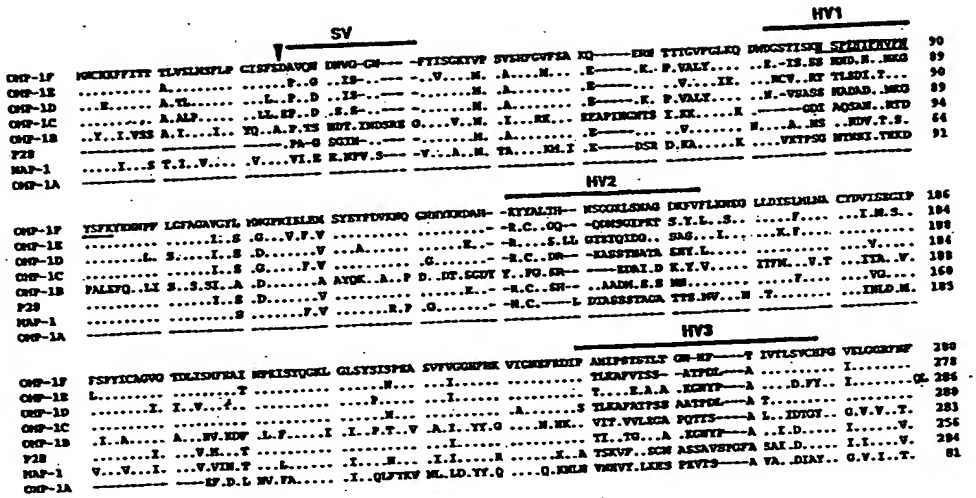

Fig. 31